

PERIODONTAL AND PULPAL CONNECTIONS FROM THE TEETH TO  
THE TRIGEMINAL MESENCEPHALIC NUCLEUS AND GANGLION IN THE  
VERVET MONKEY AND OLIVE BABOON

DR. JAMEELA HASSANALI, B.D.S. (EDIN.)

DEPARTMENT OF HUMAN ANATOMY

UNIVERSITY OF NAIROBI.

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JAMEELA HASSANALI



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SUMMARY

1. The quantitative and somatotopic aspects of periodontal/gingival and pulpal afferent connections of the mandibular and maxillary incisors, canines and molar teeth to the mesencephalic nucleus of trigeminal nerve and the trigeminal ganglion have been investigated in the vervet monkey and olive baboon using horseradish peroxidase (HRP) retrograde axonal tracing method.

2. It has been demonstrated that the periodontal proprioceptive afferent neurons of incisors, canines and molars are found predominantly in the ipsilateral caudal part of the trigeminal mesencephalic nucleus extending from the level of inferior colliculi to the trigeminal motor nucleus in pons. The incisors have significantly more mesencephalic neural connections than canines and molars. No HRP labelled pulpal mesencephalic neurons have been observed. Faintly labelled neurons have been observed bilaterally, presumably in the supratrigeminal nuclei.

3. It has been shown that the incisors and canines have a large and preponderantly ipsilateral representation in the trigeminal ganglion compared to the molars which have a sparse ipsilateral representation. The discrete periodontal/gingival and pulpal HRP labelled afferent

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neurons innervating mandibular teeth are found in the postero-lateral aspect of the ganglion and those of the maxillary teeth are found in the middle, along the dorso-ventral extent of the ganglion.

4. Present study shows that about 10% to 15% of the mesencephalic neurons (unilaterally) and 0.32% to 0.58% of trigeminal ganglion neurons have afferent connections with the periodontium of incisors, canines and molars in the monkey and baboon. The stereological analysis and cell counts in stratified serial paraffin wax sections has shown that there are bilaterally 1379-2674 and 1620-2816 mesencephalic neurons; 98073-101178 and 137250-153555 ganglion neurons in the monkey and baboon respectively.

5. The periodontal proprioceptive mesencephalic afferent connections of the anterior and posterior teeth suggest that they are involved in the modulation of the reflex effects on the jaw-opening and jaw-closing motor neurons and are thus important in the regulation of masticatory jaw movements. Moreover, a cluster of mesencephalic neurons may form a functional unit for synchronizing jaw movements during mastication. The numerous trigeminal ganglion afferent connections of the anterior teeth suggest that they have a major sensory role particularly in perception of the food bolus.

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Furthermore, the afferent connections of the anterior teeth may serve to regulate the jaw movement by providing anterior guidance during the occlusal phase of chewing. It is concluded that the connections of teeth to the ipsilateral trigeminal mesencephalic nucleus and ganglion; the connections to the interneurons of the supratrigeminal and the sensory nuclei are involved in the reflex modulation and bilateral control of jaw movements and the perception of stimulation of the teeth.

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LIST OF ABBREVIATIONS USED IN THE THESIS

I <sup>1</sup>	Central incisor
I <sup>2</sup>	Lateral incisor
C <sup>1</sup>	Canine
PM <sup>1</sup>	First premolar
PM <sup>2</sup>	Second premolar
M <sup>1</sup>	First permanent molar
M <sup>2</sup>	Second permanent molar
M <sup>3</sup>	Third permanent molar
Dec.	Deciduous
Max.	Maxillary
Mand.	Mandibular
Ophth.	Ophthalmic
Rt	Right side
Lt	Left side
Wt	Weight
Kg	Kilogram

# XIII

Pdl	Periodontal ligament
HRP	Horseradish peroxidase
HRP-WGA	Horseradish peroxidase - wheatgerm agglutinin complex
DAPI	4,6 + Diamidino - 2 - phenylindole
TMB	Tetramethyl benzidine
$V_v$	Volume density
$N_A$	Number per unit area
$N_v$	Numerical density
$N_T(\text{est})$	Estimated total number of cells
$T(\text{count})$	Total number of cell counts
V	Trigeminal nerve
Mot V	Motor nucleus of trigeminal nerve
Mes V	Mesencephalic nucleus of trigeminal nerve
Sens. V	Sensory nucleus of trigeminal nerve
Sup.Tr.N.	Supratrigeminal nucleus
L.C.	Locus coeruleus nucleus
Fig.	Figure

# XIV

msec	Millisecond
Sec.	Second
hrs.	hours
yrs.	years
H/E	Haematoxylin / Eosin
T.S.	Transverse section

## 1. INTRODUCTION

### A) GENERAL INTRODUCTORY REMARKS

1.1       Teeth are an important component of the masticatory system and the peripheral sensory information from the teeth and other masticatory components such as muscles is integrated with the central nervous system for learning control and modification of mastication as a co-ordinated function. Sensory and proprioceptive stimuli from periodontal mechanoreceptors are considered to be important peripheral sensory stimuli to the central nervous system during the function of mastication (Sherrington, 1917; Szentagothai, 1948; Darian-Smith, 1973), and appear to be an area of interest in the understanding of the complex action of mastication (Miles, 1979). Teeth, it seems, have an essential and pre-eminent role to play in this function because masticatory patterns become established only when the teeth have erupted into the oral cavity (Bosma, 1967; Moyer 1973). However, the precise role of sensory connections from the teeth, including the periodontal ligament is not fully understood (Hannam, 1976; Dubner et al., 1978; Luschei and Goldberg, 1982).

1.2       The prime function of mastication is the breaking down of food which is achieved by the co-ordinated, cyclic and rhythmic movements of the mandible, governed by the muscles of mastication and the temporomandibular

joint to enable the teeth to effectively cut or crush and chew the food within the oral cavity to a consistency that is suitable for swallowing. The masticatory function is learned, controlled and modified through the complex integration and co-ordination of the peripheral sensory and proprioceptive stimuli from the various masticatory apparatus components, the brainstem centres, cerebral and cerebellar activities and the musculo-skeletal response (Anderson and Mathews, 1976, Mohl and Drinnan, 1977, Dubner et al., 1978).

1.3. The understanding of the highly co-ordinated neuromuscular function of mastication and the role of the various components of the masticatory system is not clear. Following the work of Sherrington (1917), Rioch (1934) and Szentagothai (1948) mastication has been thought to be a neurophysiologic function mediated through the reflexes via the trigeminal system. However, masticatory function cannot be explained fully on the basis of the peripheral stimuli and reflexes alone (Goodwin and Luschei, 1974; Schaerer et al., 1966). Accumulated evidence indicates the presence of a "chewing centre" in the brainstem which is capable of elaborating cyclic patterns of muscle activity expressed in mastication (Magoun et al., 1933; Dellow and Lund, 1971; Sumi, 1971). It is evident that there is central as well as peripheral control of mastication, but it is not clear

as yet how they operate in the normal function of mastication.

1.4 Evidence mainly from studies on non-primate vertebrates indicates that the primary cell bodies of the general sensory and proprioceptive afferents from the periodontal ligament are located in the trigeminal ganglion and the mesencephalic nucleus of the trigeminal nerve (Corbin, 1940; Corbin and Harrison, 1940; Jerge, 1963a 1967; Anderson et al., 1970; Cody et al., 1972; Appenteng et al., 1982; Gottlieb et al., 1984; Capra et al., 1984; Byers, 1985, Byers et al., 1986). The primary cell bodies of the pulpal afferents are located in the trigeminal ganglion (Aker and Reith, 1980, 1981; Marfurt, 1981a,b; Capra et al., 1984). However, little information is available concerning the pulpal and periodontal proprioceptive and sensory connections in non-human primates (Kerr and Lysak, 1964; Lende and Poulos 1970; Cox et al., 1977; Chiego et al., 1980). Indeed, there is a general lack of data on the quantitative and somatotopic representation of the proprioceptive and general sensory afferents from the teeth to the mesencephalic nucleus of trigeminal nerve and the trigeminal ganglion in non-human primates. Furthermore, the question of whether or not the dental pulp has proprioceptive function has been a subject of debate (Dubner et al., 1978; Chiego et al., 1980; Capra et al., 1984; Dong et al., 1985). Moreover, the evidence

available on whether or not the proprioceptive and sensory connection is only ipsilateral or bilateral to the mesencephalic nucleus and the trigeminal ganglion appears to be conflicting (Smith et al., 1967; Cody et al., 1972; Anderson and Pearl, 1974 b; Arvidsson, 1975; Anderson et al., 1977; Fuller et al., 1979; Wilson et al., 1983; Gottlieb et al., 1984).

1.5. The aim of the present study is therefore to investigate in detail and quantify the central proprioceptive and sensory connections from the periodontal ligament and dental pulp of various teeth types in the non-human primates, namely the vervet monkey and the olive baboon (*Cercopithecinae*), using the technique of retrograde labelling of cell bodies by horseradish peroxide (HRP). It is postulated that the quantification and somatotopic mapping of labelled mesencephalic neurons and trigeminal ganglion neurons would show if the larger teeth have a greater input than the smaller teeth, and if there are any differences between the input from the anterior and posterior teeth and whether or not the input to the trigeminal ganglion and mesencephalic nucleus is ipsilateral and/or bilateral. The study further attempts to clarify the role of dental and periodontal proprioceptive and sensory innervation in the complex function of mastication by providing somatotopic and quantitative data on the



central connection of the teeth in these non-human primates.

B) HORSE RADISH PEROXIDASE METHOD FOR TRACING NEURONAL CONNECTIONS

1.6 Tracing neuronal pathways with HRP histochemistry entails the administration of a suitable enzyme preparation, its uptake and transport by neural elements, the postmortem preservation of enzyme activity, the histochemical reaction to achieve detectable reaction-product and preparation of tissue for light and electron microscopy (Mesulam, 1982).

1.7 Neuronal pathways can be traced with HRP because neurons avidly endocytose extracellular macro-molecules and because resultant endocytotic vesicles are actively transported from one part of a neuron to another. Endocytosis occurs throughout the membrane of neurons, including dendrites, perikarya, axons and their terminals (Halperin and LaVail, 1975). At the axonal terminals, endocytosis participates in the recycling of synaptic vesicles (Ceccarelli et al., 1973; Heuser and Reese, 1973; Holtzman et al., 1973). According to the short loop hypothesis, endocytotic vesicles in the nerve ending are either directly recycled into new synaptic vesicles or they first merge with elongated vacuoles and systems which in turn provide membrane for the new crop of

synaptic vesicles (Cerreccarelli et al., 1973; Heuser and Reese, 1973). Alternatively, long-loop recycling may occur through retrograde transport into the perikaryon where fusion with lysosomes and subsequent degradation may provide the components for the synthesis of new membrane which can then be conveyed by anterograde transport into the terminal region (Holtzman et al., 1973). Endocytosis also occurs along the surface of the perikaryon and dendritic branches (La Vail and La Vail, 1974; Turner and Harris, 1974). It is believed that the fate of these endocytotic vesicles is similar to that of those originating in terminals. Thus, they reach the perinuclear area, fuse with lysosomes and acquire characteristics of secondary lysosomes with the purpose of providing degradation products that can be used for the synthesis of new membranes (Mesulam, 1982). The newly formed membrane is then transported anterogradely either directly or along the elements of rough endoplasmic reticulum (Droz et al., 1975; Schwartz, 1979). There is a strict segregation between the compartments of anterograde and retrograde transport (La Vail et al., 1980). It is not clear if all the retrogradely transported endocytic organelles which originate along dendrites, perikaryal surface and axonal endings undergo complete breakdown into components once they reach the perikaryal region.

1.8        The enzyme horseradish peroxidase (HRP) is an enzyme protein and has a wide range of applications for tracing neuronal connections. The availability of crystalline HRP resulted in the determination of molecular weight around 40,000 (Cecil and Ogston 1951). Native HRP was found to consist of glycoprotein apoenzyme containing a covalently bound hematin group (Shannon et al., 1966). Although the plasma membrane constitutes an effective barrier to its free diffusion into intact cells, extracellular HRP has been reported to gain entry into neurons through a process of endocytosis (Tischner, 1977; Mesulam 1982). The membrane-delimited endocytotic vesicles which contain the enzyme are then transported along neural processes that emanate from the site of administration. (Keefer 1978).

1.9        While the HRP molecule is not itself visible, readily detectable reaction is obtained by enzymatic action at the site of administration as well as at sites of transport (Mesulam, 1982). Thus, the neuronal connections of the region injected with HRP can be determined. Research on enzymology of peroxidases has largely relied on chromogenic substances which change colour when oxidized by the peroxidase-peroxide complex (Mesulam and Rosene, 1979). Guaiac solutions which darkened upon oxidation were used as one of the initial marker for peroxidatic activity. Benzidine was used as chromogen in histochemical procedures and later used

together with nitroferricyanide which is an effective stabilizing agent for the blue reaction product (Goodpasture, 1919; Graham, 1918-1919). Straus (1957) used dimethyl-P-phenylene-diamine, a chromogen that yields a red pigment upon oxidation in his studies of HRP in renal cells. Straus (1964), described a histochemical method for the microscopic demonstration of endocytosed HRP using benzidine as a chromogen according to Gomori's (1952) recommendations. HRP containing endocytosed vesicles in liver and kidneys of rat injected with intravenous HRP were detected as a specific precipitation of blue reaction-product. Graham and Karnovsky (1966) introduced fixation with glutaraldehyde-paraformaldehyde mixture and the use of diaminobenzidine (DAB) as the chromogen which yielded a brown reaction product and that could then interact with osmium tetroxide to provide electron dense precipitate at sites of HRP activity and thus giving ultrastructural details.

1.10 The Graham and Karnovsky (1966)) procedure gained acceptance during the time when HRP histochemistry was being developed as a neuroanatomical method (La Vail and La Vail, 1974; Turner and Harris, 1974). Holtzman and Peterson (1969), reported that intact mammalian neurons can incorporate intravenously administered HRP by means of endocytosis. Zachs and Saito (1969) showed that intramuscular injections of HRP in the mouse led to the rapid uptake of the label into coated vesicles of nerve

endings in the neuromuscular junction. Hughes (1953) showed that pinocytotic vacuoles which formed at the nerve tip moved within the axon. Becker et al. (1968), were among the first researchers to notice the transport of extracellular HRP. La Vail and La Vail (1972) showed an analogous retrograde transport in central nervous system from retina to the isthmo-optic nucleus of the chick; a study which was substantiated later by La Vail and La Vail (1974) and Turner and Harris (1974). Definite demonstration of neural connection by means of transported HRP was made by Kristensson and Olsson (1971, 1973), who reported that intramuscularly administered HRP could be readily demonstrated <sup>in the</sup> brainstem and in spinal cord tissue fixed and processed with diaminobenzidine according to Graham and Karnovsky (1966) method. Retrograde transport of HRP in transected axons was also demonstrated by Kristensson and Olsson (1974, 1976).

1.11 The direct histochemical generation of a reaction-product as a precipitate at sites of HRP activity is a convenient method. The threshold for detecting HRP, the ratio of sensitivity to specificity and the resultant morphological detail are likely to vary from one method to another (Mesulam and Rosene, 1979). The distribution of reaction-product at the time of microscopic examination provides the only information for determining the site of HRP administration, the distribution of resultant transport and consequently the

pattern of neuronal connectivity. It is important to maintain enzyme activity during fixation, as the conditions of enzymatic incubations influence the amount and visibility of reaction-product that is deposited per unit of HRP activity (Mesulam, 1976; Rosene and Mesulam, 1978; Mesulam and Rosene, 1979). The resultant HRP reaction-product may be granular. It may however, be non-granular diffuse reaction product, possibly due to HRP entry through injured axon or direct intracellular administration. The diffuse HRP reaction product may also be obtained with certain chromogens such as tetramethylbenzidine (TMB), (Mesulam, 1982).

1.12 Since the absence of reaction product tended to be interpreted as indicating the absence of HRP transport, many neural connections were initially underestimated, anterograde transport was considered insignificant and some neural pathways were thought not to transport HRP (Mesulam and Rosenz, 1979). The potential for such misinterpretations are being reduced with the introduction of methods which improve histochemical sensitivity (Adams, 1977; Colman et al., 1976; Courville and Saint-Cyr, 1978; De Olmos, 1977; De Olmos and Heimer, 1977; Hanker et al., 1977; Kim and Strick, 1976; Malmgren and Olsson, 1978; Mesulam 1976, 1978; Mesulam and Rosene, 1979; Rosene and Mesulam, 1978; Mesulam 1982). Conjugation of HRP to lecithin wheat germ agglutinin has greatly improved the effectiveness of HRP as neural

tracer (Gonatas et al., 1979; Brushart and Mesulam, 1980).

1.13 It is clear now that HRP is a tracer for retrograde axonal transport but can also be employed to trace neural efferents through the process of rapid anterograde transport and that even the central sensory connections of individual peripheral organs can be determined through the process of transganglionic transport (Colman et al., 1976, Conde and Conde, 1979; Mesulam and Brushart, 1979; Kalia and Mesulam, 1978, 1980; Brushart and Mesulam, 1980). As a consequence of these developments, HRP neurohistochemistry has become, for the moment, one of the most versatile methods available to the neuroscientist for addressing virtually any question related to neural connectivity (La Vail, 1975; Mesulam 1982).

1.14 The validity of tracing neuronal connections with HRP was tested by direct comparisons with amino acid autoradiography (Mesulam and Mufson, 1980). Simultaneous intraocular injections of HRP and of tritiated amino acids yielded identical patterns of projections when alternate tissue sections were treated for HRP histochemistry and for autoradiography. In another experiment (Kalia and Mesulam, 1980), comparable results were obtained for HRP and autoradiography.



1.15 Furstman et al. (1975), were the first to demonstrate the use of retrograde axonal transport of HRP in sensory nerves from tooth pulp to the trigeminal ganglion in the rat. HRP method has since been used extensively to trace neural connections in the teeth and for somatotopic organization of trigeminal ganglion. For studying afferent neural connections from tooth pulp in the rat, (Aker and Reith, 1981; Shellhammer et al., 1984), and in the cat (Arvidsson, 1975; Anderson et al., 1977; Fuller et al., 1979; Wilson et al., 1983; Capra et al., 1984 and Henry et al., 1986), injected HRP into the pulp following cavity preparation in the teeth. In the primates, Cox et al. (1977), Kubota et al. (1979), and Chiego et al. (1980), studied neural connections from teeth pulp using HRP method. Somatotopic organization of the trigeminal ganglion of the rat (Jacquin et al., 1983a) and of the cat (Marfurt, 1981d) has been carried out by applying HRP to cut nerve endings of various branches of the ophthalmic, maxillary and mandibular divisions of the trigeminal nerve. Central projections from the trigeminal nerve branches have also been investigated using HRP method in the rat and cat (Marfurt, 1981b; Jacquin et al., 1983a Marfurt and Turner, 1983). Recently HRP method has also been used to trace afferent neural connections from the periodontal ligament in the cat (Bosley et al., 1983; Capra et al., 1984; Gottlieb et al., 1982, 1984). Afferent connections



of periodontal ligament by HRP injections into the periodontal ligament as well as onto the cut nerve endings of sensory branches innervating teeth have also been investigated in the cat (Gottlieb et al., 1984).

1.16 Researchers who have used HRP method to trace neural connections from teeth have variously discussed the limitations of the HRP method (Kubota et al., 1979; Aker and Reith, 1981; Wilson et al., 1983; Jacquin et al., 1983a, b and Gottlieb et al., 1984). The major problems encountered have been the method of HRP application to tooth pulp and in particular whether the injection should be made directly into the pulp following pulp exposure or into the pulp just before exposure. The amount of HRP solution and the mode of application such as solution, gel, paste is also variable (Griffin et al., 1979). As to how many nerve endings or receptors take up HRP molecule is also not certain (Aker and Reith, 1981). The injection of HRP into pulp may leak through the apex into the periodontal ligament (Capra et al., 1984). The amount of HRP uptake by the capsulated receptors of the periodontal ligament may also be inadequate (Gottlieb et al., 1984). The second major problem has been in determining <sup>an</sup> adequate survival period to allow retrograde transport to the neuron cell body. Some limitations in the technique of demonstrating the HRP reaction product in the neurons have also been encountered and discussed. (Jacquin et al., 1983b).

1.17 However, despite the technical problems and some limitations, HRP retrograde tracing method has been accepted as being a suitable method for tracing neural connections from teeth. It is generally agreed, however, that in order to realize the full potential of HRP histochemistry the mode of enzyme administration must be such that uptake and subsequent transport are confined to the area under investigation and its connections (Jones and Leavitt, 1974; Jones, 1975). It is also desirable to ensure maximum uptake and transport along these pathways by avoiding experimental conditions such as high doses of anaesthetic agents which may interfere with transport (Rogers et al., 1980). Furthermore, it is necessary to select a survival interval which allows sufficient time for transport and subsequent accumulation of HRP without excessive degradation by lysosomal enzymes (La Vail and La Vail, 1974; Turner and Harris, 1974; Mesulam and Mufson, 1980). Moreover, the fixation must offer adequate tissue preservation and enzyme localization without excessive loss of HRP activity (Rosene and Mesulam, 1978; Courville and Saint-Cyr, 1978). Lastly, it is also necessary that histochemical procedures should provide a high level of sensitivity and specificity and finally the subsequent histochemical procedures in preparing the tissue for microscopic examination should preserve the location and visibility of the reaction product (Mesulam, 1982).

C) THE ROLE OF DENTAL SENSORY MECHANORECEPTORS AND  
PROPRIOCEPTORS IN MASTICATION

1.18 According to Sherrington (1947) the term proprioception refers to information provided about the movements and position of the body and its parts by receptors in muscles, tendons and joints. Such receptors are not generally considered to produce conscious sensations or related to conscious control. The stimuli to the receptors are given by the organism itself and these receptors called proprioceptors are concerned with sensibilities of position, pressure and sense of movements. In muscles and tendons, there are proprioceptors such as muscle spindles and golgi tendon organs which are stimulated by changes in the tension and/or length of muscles and the resulting nerve impulse is transmitted to the central nervous system where muscle activity is co-ordinated and regulated.

1.19 There are many receptor systems in and around the mouth which provide information on which the control of muscle activity and jaw movement is based (Taylor, 1981). The mechanoreceptors of the periodontal ligament, for example are generally thought to provide a substantial part of this information (Anderson et al., 1970; Dubner et al., 1978, Luschei and Goldberg 1982; Hannam, 1976, 1982). Subsequently, therefore, several types of nerve endings have been described within the periodontal

ligament, varying from simple free nerve endings to more specialised encapsulated and unencapsulated endings (Lewinsky and Stewart, 1937a, b; Bernick, 1952, 1957; Fallin, 1958; Kizior et al., 1968; Pimenidis and Hinds, 1977; Harris and Griffin, 1974 a,b; Griffin and Harris, 1974 a,b; Hannam, 1982; Berkovitz et al., 1983; Byers, 1985; Byers et al., 1986). Simple, compound and complex encapsulated nerve endings have been classified histologically (Griffin and Harris, 1974 ab), while slowly, rapidly and spontaneously adapting receptors have been classified electrophysiologically (Pfaffmann, 1939 a,b; Ness, 1955; Linden, 1978) in the periodontal ligament. Laminated nerve terminals in periodontal ligament of rat incisor, cat and crocodile teeth have been described (Bonnand et al., 1978; Berkovitz and Sloan, 1979; Berkovitz et al., 1983). Thus most of the periodontal receptors may be regarded as mechanoreceptors as they respond to the compressive forces borne by teeth during mastication.

1.20 The light microscopic studies (Lewinsky and Stewart, 1937ab; Rapp et al., 1957; Bernick, 1959, Bernick and Levy, 1968) and electron microscopic studies (Griffin, 1972, Harris and Griffin, 1974a,b; Griffin and Harris, 1974ab) have shown that both large and small diameter nerve fibres supply the periodontium. The large myelinated fibres correspond to the group I fibres of mean diameter, 8-14  $\mu\text{m}$ , while the small diameter fibres

are group III myelinated fibres 4-9  $\mu$ m. Some small fibres are unmyelinated and may be autonomic as they are found in close association with blood vessels. However, some of the unmyelinated fibres in human teeth terminate throughout the periodontal ligament with a receptor type nerve ending showing <sup>the</sup> presence of mitochondria which indicates that they may be considered to be part of <sup>the</sup> receptors system. These are suggested to be afferent fibres (Harris and Griffin, 1968; Berkovitz and Shore, 1978). The large and small myelinated nerve fibres have been studied considerably and various types of nerve terminals described. Some studies describe non-specialized nerve endings such as free nerve endings, while others describe organized structures. They may be encapsulated simple, complex or compound endings, twisted and convoluted loops or networks and coiled knobs and spindle-like endings (Lewinsky and Stewart 1937ab; Rapp et al., 1957; Simpson, 1966; Griffin and Harris, 1968, 1974 a,b). The various types of nerve terminals are thought to perceive pain, pressure, touch, light forces and temperature stimuli.

1.21 Anatomically, periodontal receptors are classified as free nerve endings, encapsulated or unencapsulated receptors which in keeping with laminated endings are thought to be mechanoreceptors. Van der Sprenkel (1936) described small end-rings completely surrounded by a periterminal reticulum in the periodontal ligament of

young mice. These end-rings were terminations of myelinated fibres and lay on collagen fibre bundles located near the alveolus, and their function was to subserve pressure. In the human periodontium, Lewnisky and Stewart (1937 a) found small round endings. In the cat Lewinsky and Stewart (1937 b) found that the large fibres confined to the peripheral part of the ligament have specialized end-organs while the finer fibres break up into fine arborization without terminal organs. They suggested that the large myelinated fibre with special end-organs were associated with pressure and tactile sensations, while the finer myelinated and unmyelinated fibres are associated with pain perception. Simpson (1966), in human extracted teeth described the large fibres terminating as fine unmyelinated endings with a knob-like enlargement, or myelinated fibres terminating in an irregular ending. In the guinea-pigs and marmosets Bernick and Levy (1968) found only fine nerve endings in the upper two thirds of the root. These were probably pain and nociceptive receptors while in the lower third of the root, nerves terminated in club-like structures, possibly for pressure and deep touch perception. Kizior et al., (1968), in the cat found large ovoid fibres surrounded by connective tissue capsules at the apical third, possibly suggesting that they respond to light forces while the small endings throughout the ligament probably act as pain receptors. Mei et al., (1977)

described periodontal mechanoreceptors in the cat involved in pain perception. Fallin (1958) described two types of the nerve endings which were thought to be presumptive mechanoreceptors giving information on tension of collagen bundles and change in position of tooth during mastication. Fallin (1958) further suggested that their stimulation may be a source of severe pain. Byers (1985) and Byers et al. (1986), described four types of Ruffini-like unencapsulated receptors associated with connective tissue in the periodontal ligament of <sup>the</sup>rat and <sup>the</sup>cat.

1.22. Griffin and Harris (1968); Griffin and Spain (1972); Harris and Griffin (1974ab) described nerve endings of fine myelinated and unmyelinated fibres in developing and functional human periodontal ligament with electron microscopy. Griffin and Spain (1972) described the fine structure of human periodontal nerve plexuses with fusiform, oval and end-ring and encapsulated endings. Griffin (1972) described the structure of end-rings in human periodontal ligament at the light microscopic level appearing as clusters of encapsulated nerve endings surrounded by dense collagenous tissue. At the electron microscopic level, nerve endings are seen containing one or more myelinated and/or unmyelinated fibres. The unmyelinated fibres either partially or completely encircle myelinated fibres. The myelinated fibres of 2-3 $\mu$ m in diameters are enclosed in Schwann cell



cytoplasm and are separated from unmyelinated fibres by sparse endoneurium. The nerve endings were separated from the extra neural tissue by capsular cells, tissue or lymph space and collagen fibres. The unmyelinated nerve fibres terminated either as minute axons enclosed in Schwann cell bays or as partially exposed axons (Harris and Griffin, 1974a,b). However, Byers (1985) and Byers et al. (1986), have described unencapsulated mechanoreceptors and free nerve endings similar to those described by Everts et al. (1977), and discussed and disputed the presence of encapsulated endings described by Griffin and Harris (1974 ab).

1.23 On a physiological basis, Pfaffmann (1939 a,b), Ness (1954) and Sakada and Kamio (1971) described three types of periodontal mechanoreceptors in studies on rabbit and cat teeth, namely, slowly adapting, rapidly adapting and spontaneously discharging units. The slowly adapting receptor units were shown to give a train of impulses throughout the mechanical stimulation of the tooth crown and therefore respond to phasic and sustained forces. The rapidly-adapting receptors give one or two impulses at the 'on' or at the 'off' of the stimulus and conceivably respond while the force is being applied but do not continue to fire to a sustained force. The spontaneously discharging units emit, in the absence of overt stimulation, a continuous steady stream of impulses whose frequency can be altered by the appropriate



manipulation of the incisor and of the mandibular teeth. Ness (1954) further showed that the slowly adapting receptors were predominant, while the spontaneously discharging units were few. The effects of direction of a stimulus to these receptors seems to be such that the nearer a stimulus is to the most sensitive direction of a receptor, the response frequency will be greater, threshold will be less, discharge will be longer and the first impulse will be initiated earlier (Ness, 1954). Mei et al., (1975), described similar functional characteristics of the periodontal mechanoreceptors in the cat. The receptors are activated when food is placed between the teeth and most of them are found in the canines and in the apical part of the periodontium. In single unit recordings from human inferior alveolar nerve, the slowly adapting periodontal afferents similar to those in animals have been described (Johansson and Olsson, 1976).

1.24 Griffin and Harris (1974<sub>ab</sub>) classified the mechanoreceptors as simple or discrete, consisting of an encapsulated ending with a single myelinated nerve fibre and the compound mechanoreceptors having few unmyelinated nerve endings which surround and encircle the adjacent myelinated fibres. A clustering of compound terminals is termed a complex mechanoreceptor. Griffin (1972) suggests that the compound mechanoreceptors may be either rapidly adapting, slowly adapting or spontaneously

discharging and might be coupled to the tissue by viscous and elastic coupling. In the case of elastic coupling to the tissue, their terminals would follow the mechanical distortion so that adaptation would be slow, whereas, if the receptor was viscously coupled to the tissue, slipping would result in rapid adaptation. Elastically coupled receptors would then discharge if the threshold was exceeded, whilst viscously coupled receptors would require a critical rate of application of stimulus. As regards the periodontal encapsulated endings, the capsule appears to be fine, consisting of two to three layers of capsular cell processes immediately adjoining periodontal fibrous tissue. Therefore, if the capsule controlled the ending, it would seem to be under the influence of the predominantly fibrous periodontal tissue which according to Hannam (1969 ab, 1976) exerts a constant tension on the ending resulting in a spontaneous discharge. However, there is a tissue or lymph space between the ending and the capsule proper and thus there may be a time lapse before the ending proper would return to its former position. Thus, it may be that the compound mechanoreceptors are rapidly adapting. Studies of Fallin (1958), Everts et al., (1977), and Byers (1985) show that some nerve endings arborize within the ligament fibres and suggest that these may be slowly adapting highly sensitive receptors as they would be deformed most easily while those endings in the loose connective tissue may be

rapidly adapting. Although the mechanoreceptors have been linked to the functionally distinct rapidly adapting, slowly adapting and spontaneously discharging receptors indicated from physiological studies of periodontal afferents, the actual correlation is still not clear.

1.25 The studies carried out on the distribution of nerve endings in the periodontal ligament by Bernick (1957); Byers and Matthews (1981); Bernick and Levy (1968); indicates that the termination of the large fibres occurs predominantly in the apical region of the cat periodontium as opposed to the more general distribution of the unmyelinated fibre endings in the upper part. Byers and Holland (1977) using autoradiographic labelling of trigeminal ganglion rat molar teeth showed nerve endings in gingival and junctional epithelium and found a low level of apical periodontal labelling. Kubota and Osnai (1977) found higher density of free endings in Japanese shrew mole in the apical regions of the teeth than the cervical region and suggested that the difference in results may be due to species difference. It was also thought that the low level of apical periodontal labelling observed following the axonal tracing from the trigeminal ganglion in rat in the study of Byers and Holland (1971) was due to the afferent cell bodies of most of the periodontal receptors

being located in the mesencephalic nucleus rather than the trigeminal ganglion.

1.26 The electrophysiological studies of Mei et al., (1975), and Cash and Linden (1981, 1982) found the receptors to be located in the apical and lingual aspect of the periodontal ligament of the canine tooth of the cat. Electrophysiological and anatomical studies indicate the presence of periodontal afferent neurons in the mesencephalic nucleus of trigeminal nerve as well as the trigeminal ganglion (Corbin and Harrison, 1940; Kerr and Lysak, 1964; Cody et al., 1972, Darian-Smith, 1973; van Steenberghe, 1979; Gottlieb et al., 1984). Byers (1985) and Byers et al. (1986), have attempted to elucidate the position and structure of the periodontal receptors which have the afferent cell bodies in the mesencephalic nucleus of trigeminal nerve and in the trigeminal ganglion using radio<sup>active</sup> labelled amino acid. The receptors of the myelinated mesencephalic axons were found to be unencapsulated Ruffini-like mechanoreceptors located close to the root apex while those of the trigeminal ganglion were unencapsulated Ruffini-like mechanoreceptors and free nerve endings. The ganglion receptors were about 5 times greater next to the lower third of the root than in the upper two thirds or at the apex. Functionally, the ganglion receptors that were found within the ligament fibres are thought to be slowly adapting while the mesencephalic receptors which were mainly within the

loose connective tissue may be rapidly adapting (Byers, 1985; Byers et al., 1986).

1.27 Much remains to be known, as to which receptors perceive which modalities of sensation. It is, however, generally assumed from histological studies of periodontal innervation, that the large myelinated fibres and their terminations (receptors) contribute to tactile sensations of the tooth whereas the small unmyelinated fibres are implicated in pain and nociception (excluding the autonomic fibres related to blood vessels). The structure of the mechanoreceptor suggests a function which might account for the physiologically adapting properties, but it is still premature to assign a role for each receptor in jaw reflex functions as it is to implicate unmyelinated fibres in pain perception. (Harris and Griffin, 1975; Byers, 1985)

1.28 The role of the nerve endings in the dental pulp has also been considered in previous studies. The small diameter myelinated and unmyelinated nerve fibres have been described in the mature pulp (Brashear, 1936; Bernick, 1952; Brookhart et al., 1953; Fehrer et al., 1977; Kubota et al., 1982). Some of the unmyelinated fibres may be sympathetic and parasympathetic while the small diameter myelinated fibres may be involved in pain perception (Avery et al., 1978, 1980 ; Avery, 1981).

1.29 In the light microscopic study of human pulp, three types of nerve endings; namely, one of a complex glomerular nature, another showing branched terminals especially in subodontoblastic regions and third associated with blood vessels have been described by Seto (1972). Harris and Griffin (1968) also described three types of receptors in an electron microscopic study of pulp. One of these was related to blood vessel, second, an unmyelinated nerve ending and a third category of a beaded fibre that ended as an axonal expansion. The beaded endings may be derived from myelinated nerves and comparable to the beaded endings that arise from the subodontoblastic fibres seen at light microscope level (Fearnhead, 1967).

1.30 Kubota and Kubota (1959) observed the relation between nerve endings and blood vessels in the human tooth pulp. Sensory nerve endings and coiled structures closely related to the blood vessels may be correlated with the regulation of blood circulation in the pulpal vessels and with the perception of pain of from dental pulp. A number of cytochemical labelling methods have demonstrated that there are, in addition to sensory components, sympathetic primary afferents and possibly parasympathetic endings in the pulp. Adrenergic nerve endings in the dental pulps of mouse molars were shown by Avery et al. (1980). Byers and Matthews (1981), and Byers (1983) studied the location of sensory nerve

endings in the cat and monkey dentine and pulp using autoradiographic method and showed that the labelled axons were unbranched in the root and arborized in the crown to end among odontoblasts and many adjacent dentinal tubules. Chiego et al. (1980), following labelled HRP injection into pulp showed labelling of neurons in the trigeminal, superior cervical sympathetic and otic ganglia as well as mesencephalic nucleus of trigeminal nerve. Pimenidis and Hinds (1977) described apparent corpuscular sensory receptors in the pulp in an autoradiographic study of innervation of the rat molar teeth. The presence of corpuscular receptors suggests that the dental pulp may be responsive to sensory modalities other than pain, particularly touch and pressure (Dubner et al., 1978).

1.31 In subsequent studies, four types of nerve fibre endings have been demonstrated in the pulpal/dentine zone (Gunji, 1982). These nerve endings are those of marginal pulpal fibres, simple pre dentinal fibres, complex pre dentinal fibres and dentinal fibres upto 100um from the odontoblast-pre dentinal border. The free endings are thought to conduct pain sensation, while the endings located adjacent to the odontoblast processes are considered to form a mechanoreceptive complex and conceivably play a role in dentine sensitivity and dentine formation.



1.32 The evidence derived from the foregoing review seems to indicate the presence of free, complex unencapsulated and simple, compound and complex capsulated nerve endings (discrete and compound units) in the periodontal ligament. Furthermore, it is believed that the encapsulated and unencapsulated endings subserve a mechanosensitive function. The mechanoreceptive endings are more predominant in the apical region (Anderson et al., 1970; Harris and Griffin, 1974 a,b; Byers, and Mathews, 1981; Byers 1985; Byers et al., 1986). The physiologic characteristic of the mechanoreceptors have <sup>also</sup> been studied though it is difficult to assign a functional role to the receptors from the microscopic structure. It is conceivable, however, that the interpretation of sensation, when forces are applied to the teeth, must take into account the central organisation of the variety of neurons concerned with relaying tactile information to the cortical region. This, in turn, makes any comparison between sensory experience and a single peripheral nerve fibre response, a complex affair (Hannam, 1976). Moreover, it is not possible to differentiate the mechanoreceptor nerve terminals that originate from the cell bodies in the trigeminal ganglion or the mesencephalic nucleus. Periodontal afferent cell bodies are located in the trigeminal ganglion and mesencephalic nucleus while those of dental pulp are located in the



trigeminal ganglion (Cody et al., 1972, Aker and Reith, 1981; Appenteng et al., 1982; Capra et al., 1984; Gottlieb et al., 1984).

D) NEURAL CONNECTIONS FROM THE TEETH TO THE TRIGEMINAL GANGLION AND TRIGEMINAL BRAINSTEM NUCLEAR COMPLEX.

1.33 There are several aspects of the trigeminal sensory structures and functions which are uniquely different from the spinal system, particularly the trigeminal proprioceptive aspects (Miles, 1979; Kruger and Young, 1981). The sensory modalities subserving oral and facial function are more complex and specialized than in any other region of the body (Dubner et al., 1978). The innervation of the teeth consists principally of sensitive mechanoreceptors located in the periodontal ligament subserving a crucial role in regulating force and direction of mastication. The functional aspect of the innervation of dental pulp is a sort of an enigma, in that the dental pulp receptors are thought to be activated by minimal mechanical, thermal or chemical stimuli and result in the sensation of pain (Dubner et al. 1978).

1.34 The neural basis of mastication is mainly the trigeminal system. The peripheral component consists of sensory and motor roots and the trigeminal ganglion which contains cell bodies of general sensory afferents from

the head region. The central component consists of the brainstem nuclei; namely, the motor nucleus, chief sensory and spinal nuclei which contain secondary neurons for modalities of general sensation such as touch, pressure, pain and temperature and the mesencephalic nucleus which contains primary neurons of proprioceptive afferents (Darian-Smith, 1973).

1.35 The trigeminal nerve which makes up the peripheral component is made up of the portio major or sensory root and portio minor or motor root. Rand (1969) designated the name portio-intermediate for a group of intermediate fibres. The portio major consists of afferent peripheral and central fibres of the trigeminal ganglion made up of sensory rootlets which are both myelinated and unmyelinated. Young (1977), estimated about 125,000 fibres in <sup>the</sup> human sensory root of which 50% are myelinated, while the unmyelinated or C-fibres are about 50-80%. The peripheral distribution of the portio major is through the sensory fibres of the ophthalmic, maxillary and mandibular nerves which terminate in various receptors in the skin and connective tissue as well as the mucous membrane of the eye, nose and the oral cavity, including the teeth. The peripheral distribution of the three divisions is to the respective ophthalmic, maxillary and mandibular regions of the head. The portio minor contains the peripheral axons from motor neuron somata in the trigeminal motor nucleus which extend to the muscles

of mastication. The portio minor exits the pons rostral to the portio major and takes a course diagonally across the ventral aspects of the latter to pass medial to the trigeminal ganglion and join the mandibular division for distribution to the muscles of mastication. Some sensory fibres are also thought to be contained in the motor root as unmyelinated fibres have been found in portio minor (Kruger and Young, 1981). The motor root also carries the proprioceptive afferent fibres from muscles of mastication (Corbin and Harrison, 1940; Ryu and Kawana 1985). The intermediate root comprises a variable number of fine nerve bundles (Gudmundsson et al., 1971). These have been thought to be either pure motor in function or sensory, being activated by jaw movement (Pelletier et al., 1974). There is no anatomical or physiological evidence to support the contention that the afferent fibres in the intermediate root subserve a specific sensory modality (Rand, 1969). Rather, some of the fibres may subserve a proprioceptive function related to jaw or muscle movement, while others may serve as cutaneous sensory receptors.

1.36 The trigeminal ganglion is made up of the sensory primary neuron cell bodies which are essentially similar to the spinal root ganglia (Lieberman, 1976). The large pseudounipolar or bipolar neuron cell bodies are entirely surrounded by closely apposed satellite cells (Dixon, 1963 a; Pineda et al., 1967). In mammals, the

trigeminal ganglion neuron initial axon or stem process, immediately after arising from the soma forms a complex and tortuous intracapsular structure before dividing into the peripheral and central branches (Pineda et. al., 1967; Lieberman, 1976). It has been demonstrated that a somatotopic organisation based on the peripheral location of the receptive field occurs at all levels in the pathways of primary afferent neurons (Darian-Smith, 1973; Drew, 1980; Marfurt, 1981a,b; Jacquin et al., 1983 a,b). Thus, ganglion cells innervating a common peripheral territory are grouped close to one another within the trigeminal ganglion and the central processes of such cells remain close to one another as they approach the brainstem. In other words, the trigeminal ganglion is roughly divisible into three major regions which are associated with each of the main peripheral divisions, namely, ophthalmic, maxillary and mandibular (Kerr, 1963). This somatotopic pattern has been confirmed by electrophysiological studies although discontinuities in the sequential pattern may be encountered (Kerr and Lysak, 1964). A similar pattern of discontinuities has been noted in experiments in which ganglion cells were retrogradely labelled by applying HRP to individual nerves (Marfurt, 1981a; Kruger and Young, 1981). Retrograde labelling of the ganglion from the central axons of <sup>the</sup> sensory root is more homogeneous and complete, but a similar pattern of patchiness can be seen with

retrograde labelling following HRP injection into separate sections of the sensory trigeminal nuclear complex. The proportion of labelled cells projecting to the principal sensory nucleus of trigeminal nerve does not appear to exceed those to subnucleus caudalis and both projection zones receive input from large and small ganglion cells (Kruger and Young, 1981).

1.37 Both anatomical and physiological techniques have been used in an attempt to establish the somatotopic organisation of the trigeminal ganglion and its connections with the trigeminal brain-stem nuclear complex. Combined degeneration studies (Mazza and Dixon, 1972; Gregg and Dixon, 1973), and retrograde HRP studies (Arvidsson, 1975; Anderson et al., 1977; Cox et al., 1977; Fuller et al., 1979; Aker and Reith, 1980, 1981; Chiego et al., 1980; Marfurt, 1981 a,b; Jacquin et al., 1983ab; Wilson et al., 1983; Shellhammer et al., 1984; Henry et al., 1986) in the rat, cat and monkey have revealed that although there is evidence of somatotopic organisation of the trigeminal ganglion in the mandibular, maxillary and ophthalmic compartments, much remains to be known about the exact somatotopy of the neurons innervating maxillary and mandibular teeth. In the cat, for example, HRP studies (Anderson et al., 1977; Arvidsson and Gobel, 1981; Marfurt, 1981a; Wilson et al., 1983) and electrophysiological studies (Beaurdeau and Jerge, 1968; Darian-Smith et al., 1965,

the fibres in  
 ; Lisney, 1978) have shown that the somata of the  
 mandibular nerve which innervate the lower third of the  
 face and jaws, as well as the ventral half of the oral  
 cavity occupy the postero-lateral and ventral portion of  
 the trigeminal ganglion and that the cell bodies of the  
 ophthalmic branch supplying the dorsal third of the face  
 are located antero-medially and dorsally. The somata of  
 the fibres in  
 the maxillary branches which innervate the maxillary  
 region are situated in the middle. According to Jacquin  
 et al. (1983a), the cell bodies of the nerves that  
 innervate the posterior and/or lateral portion of the  
 head and face are located in the dorsal regions, while  
 the somata of the nerves that supply the more rostral  
 oral and peri-oral regions have a predominantly ventral  
 location.

1.38 There is also conflicting evidence on whether or  
 not there exists any peripheral cross-innervation of the  
 teeth contralateral to the trigeminal ganglion. Anderson  
 et al. (1977), Fuller et al. (1979), Fuller and Wilson, (1980)  
 and Wilson et al.  
 (1983), in the cat, Furstman et al. (1975), and  
 Shellhammer et al. (1984); in the rat and Cox et al.  
 (1977), and Chiego et al. (1980), in the monkey, found  
 conflicting evidence using HRP retrograde method. Byers  
 and Matthews (1981) found scarce evidence of cross  
 innervation in the cat trigeminal ganglion, but  
 electrophysiological studies have denied the presence of

cross-innervation in <sup>the</sup> canine tooth (Matthews and Lisney 1978; Lisney, 1978).

1.39 Several workers have attempted to carry out a cell count of the trigeminal ganglion but there is no conclusive data on the total cell counts in the cat, rat and monkey (Blinkov and Glezer, 1968; Altman and Dittmer 1972). Total cell counts ranging from about 23,000 to 46,000 have been reported in the trigeminal ganglia from four rats (Aldoskogius and Arvidsson, 1978).

1.40 Studies conducted on the brainstem trigeminal nuclear complex comprising the motor nucleus, the mesencephalic, chief and spinal sensory nuclei have revealed that the size, extent and complexity of these nuclei exceeds that of all other cranial nerves and several aspects of its subdivision remain unresolved (Marfurt, 1981b; Jacquin et al., 1983a).

1.41 The motor nucleus consists of the groups of alpha and gamma motor neurons found in the pons innervating the muscles of mastication (Dubner et al., 1978). Studies in the rat (Mizuno et al., 1975; Limwongse and De Santis, 1979), in <sup>the</sup> cat (Mizuno et al., 1975; Batini et al., 1976) in <sup>the</sup> monkey (Ibrahim and Leong, 1979), in <sup>the</sup> rabbit (Matsuda et al., 1978) and in <sup>the</sup> guinea pig (Tal, 1980), have revealed that the jaw closing motor neurons tend to lie dorsal and lateral to the jaw opening moto-neurons. In addition, Mizuno et al., (1975); Limwongse and De Santis



(1979); Szekely and Matesz (1982); Jacquin et al., (1983); reported a group of HRP labelled jaw opening motor neurons as a subnucleus in the caudo-ventromedial motor nucleus of trigeminal nerve in the rat.

1.42 The sensory trigeminal complex is generally recognized to include the chief principal sensory nucleus and its extensive caudally contiguous spinal nucleus (Kruger and Young, 1981). The latter is divided into subnuclei oralis, interpolaris and caudalis. The chief nucleus has been considered to be the homologue of the dorsal column nuclei and the principal contributory to the trigeminal lemniscus joining the medial lemniscus in a massive projection to the ventrobasal thalamus. (Kruger and Young 1981). The nucleus has been shown to receive sensitive cutaneous mechanoreceptor inputs from all three divisions of the trigeminal nerve (Kruger and Young, 1981). Electrophysiological mapping studies of single neurons of this nucleus have revealed an orderly somatotopic pattern in the several species studied, with the mandibular division represented dorsally and dorsomedially, the ophthalmic and maxillary divisions ventrally and laterally (Marfurt, 1981b; Jacquin et al., 1983a). This sequence has been reported throughout most of the length of the descending spinal nucleus of the cat and monkey (Kruger and Michel, 1962; Kerr et al., 1968). However, an onion-peel rostrocaudal sequence of oral and



peri-oral fields has been shown in the subnucleus caudalis (Yokota and Nishikawa, 1980; Marfurt, 1981b). Evidence does <sup>also</sup> exist that the input to the chief nucleus is derived from the large trigeminal ganglion cells conducting via large fast axons subserving a variety of mechanoreceptor functions with no contribution from specific nociceptors or thermoreceptors (Kirkpatrick and Kruger, 1975).

1.43 The spinal nucleus is thought to consist of the subnucleus oralis which constitutes a slender ventral continuation of the principal trigeminal nucleus, subnucleus interpolaris and subnucleus caudalis Olszewski, (1950). Like the principal nucleus, it has a similar somatotopic tactile organisation and a projection to the thalamus, although the neuron population is morphologically different from the principal nucleus (Kruger and Young, 1981). The spinal subnucleus interpolaris is a distinct entity, contiguous with the caudal pole of the subnucleus oralis in the region rostral to the medullary obex. It also has a somatotopic organisation and projects to the thalamus. However, it differs from the subnucleus oralis in being very expanded and in having a large projection to the cerebellum (Cheek et al., 1975). The spinal subnucleus caudalis is large and descends down to the upper cervical spinal level, and resembles the dorsal horn of the spinal cord. It has a dominant and somatotopic input like the rest of the

spinal nucleus. It differs from these subnuclei in that it receives a segregated input from specific nociceptors and thermoreceptors (Mosso and Kruger, 1973; Prince et al., 1976). There, the nucleus proprius neurons respond to a wide dynamic range of inputs and also pain perception (Mayer et al., 1975). Large numbers of the nucleus proprius neurons are not labelled with HRP after a thalamic injection unlike the principal, oral and interpolaris nuclei (Kruger et al., 1977; Shigenaga et al., 1979).

1.44 The supratrigeminal nucleus described by Lorente de No (1922) is a dorsomedial component of the contiguous principal trigeminal nucleus and apparently lacks cutaneous input. Its position, at the caudal termination of the mesencephalic nucleus suggests an involvement in proprioceptive function and this is supported by electrophysiological findings of muscle spindle, articular and periodontal receptor input (Jerge, 1963b). Separation of the dorsomedial tactile component of the principal nucleus from the supratrigeminal "proprioceptive" nucleus is well illustrated by Mizuno (1970) and because of its position between the main sensory, motor and mesencephalic nucleus, it is sometimes called the intertrigeminal nucleus (Taber, 1961). The interneurons of the supratrigeminal nucleus have been thought to be involved in jaw-opening reflex (Kawamura, 1974). The location of the supratrigeminal

nucleus in the pons is about 500  $\mu\text{m}$  above the trigeminal motor nucleus and the firing of the interneuron by mandibular nerve stimulation is about 4msec (Kawamura, 1974). The background activity of the supratrigeminal neurons shows that steady impulses flow into this nucleus (Takata and Kawamura, 1970). Mizuno et al. (1978) have also suggested that interneurons are found in the supratrigeminal nucleus. Further study of Mizuno et al. (1983) has shown the premotor nuclear connections of the motor nucleus of <sup>the</sup> trigeminal nerve to the supratrigeminal nucleus. Since there is no definite large projection from the supratrigeminal nucleus to the cerebellum (Watson and Switzer, 1978); the functional status of the supratrigeminal nucleus remains uncertain although it may represent a "proprioceptive" aggregation of interneurons as suggested by Torvik (1957), Jerge (1963b) and Mizuno et al. (1978). Jerge (1963b) reported 3 groups of units of the supratrigeminal nucleus. The first group is of interneurons activated by pressure stimulation of intraoral structures such as teeth, gingivae and palate. The other two groups are related to the jaw-opening movements, one group shows an increase in the discharge frequency and the other is inhibited by jaw-opening. Gura et al. (1972), reported that there are two groups of supratrigeminal neurons, namely, those activated only by sensory afferents which are thought to act as excitatory interneurons for digastric motor neurons and another

group which is responsible for inhibitory effects on both jaw-opening and jaw-closing motor neurons. The suggestion of Nakamura et al. (1973a,b) that supratrigeminal nucleus contains the final inhibitory neurons in the neural chain for the peripherally evoked short-latency inhibition of jaw-closing moto-neurons supports this concept. Kidokoro et al. (1968), and Sumino (1971) also suggested that the interneurons within the supratrigeminal nucleus were inhibitory to jaw-closing muscle activity and are concerned with jaw-opening reflex. Distinguishing between 'interneurons' and 'sensory' neurons requires very stringent discrimination, although there is evidence of interneuron role of the supratrigeminal nucleus for jaw-opening reflex and bilateral coordination of oral motor behaviour (Kidokoro et al., 1968, Uchizono, 1975; Rokx et al., 1986b).

1.45 The various subdivisions of the sensory trigeminal brainstem nuclei were generally assumed to function relatively independently. Primary afferent fibres subserving specific sensory submodalities were believed to synapse on second order neurons in the associated trigeminal brainstem nuclei which perhaps after relay via one or more interneurons projected to the appropriate thalamic nuclei (Kruger and Young, 1981). However, ascending intranuclear pathways, originating in the nucleus caudalis and terminating within the more rostrally located nuclei have functionally significant

interactions as shown by the demonstration of an ascending intranuclear pathway originating in nucleus caudalis (Stewart and King, 1963). Inputs to nucleus caudalis have also been demonstrated from the contralateral nucleus caudalis (Kerr, 1972). The intranuclear organisation of the sensory trigeminal complex is poorly understood and may be crucial to understanding the role of each subnucleus which is extensively interconnected by deep bundles (Gobel and Purvis, 1972). Electrophysiological studies have demonstrated that the nucleus caudalis maintains a tonic presynaptic hyperpolarizing influence on trigeminal primary afferent preterminal in the main sensory nucleus oralis (Scibetta and King, 1969).

1 46 There is conflicting information in the available literature on the central sites of representation for dental and oral structures (Nord and Young, 1975; Sessle and Greenwood, 1976; Nord 1976; Anderson et al., 1977; Gobel and Bink 1977; Dubner et al., 1978; Nord and Rolince, 1980). Physiological and anatomical studies have demonstrated a mainly rostral or a mainly caudal distribution of the central fibres of the trigeminal ganglion in the sensory nuclei in the brainstem (Grant and Arvidson, 1975; Westrum et al., 1976; 1984; Grant et al., 1979; Johnson and Westrum, 1980). Using HRP method, Westrum et al. (1981), studied the precise sites of representation in the brainstem, for specific teeth and

their periodontal structures. They found that <sup>the</sup>maxillary canines had <sup>a</sup>greater number of labelled terminals than the mandibular canines, but overall there was much more extensive rostro-caudal ipsilateral central representation in the trigeminal brainstem nuclear complex than had been previously reported for individual teeth. Marfurt and Turner (1984) studied the central projections of tooth pulp afferents in the rat using HRP method and showed representations in the principal and rostral parts of pars oralis. Sessle and Greenwood (1976) and Lisney (1978) showed central connections of tooth pulps in the cat to the main sensory and nucleus oralis and caudalis using electrophysiological studies. Marfurt (19891b), Matesz (1981) and Jacquin et al. (1983a), have revealed neural connections from trigeminal branches to the supratrigeminal nucleus and other brainstem nuclei.

#### E) MESENCEPHALIC NUCLEUS OF TRIGEMINAL NERVE

1.47 The mesencephalic nucleus of the trigeminal nerve is a unique group of primary cell bodies of proprioceptive afferents from the head region located within the central nervous system. The nucleus consists of characteristically large (30-60µm) and small to medium (10-30µm) sized unipolar neurons having a centrally placed nucleus with a distinct nucleolus and cytoplasm with Nissl granules (Weinberg, 1928; Sheinin, 1930; Capra

et al., 1985). The mesencephalic neurons are arranged as a crescent shaped band of cells situated at the periphery of the aqueductal gray matter, and extends from the midbrain at the level of the superior colliculi to pons at the level of the motor nucleus of the trigeminal nerve (Weinberg, 1928). Many of the neurons of the mesencephalic nucleus show a feature of cell clustering of large and/or small neurons, in clusters of 3 to 8 cells (Hinrichsen and Larramendi 1969, 1970; Sivanandasingham and Warwick, 1976). The peripheral distribution of the nucleus is mainly with the motor root of the trigeminal nerve to the muscles of mastication and with some sensory branches to the teeth, gums and hard palate (Corbin and Harrison, 1940; Jerge, 1963 a, Ryu and Kawana, 1985). Functionally, the mesencephalic nucleus has been associated with proprioceptive input from the muscles of mastication, teeth and possibly also the extraocular muscles (Corbin and Harrison, 1940; Jerge, 1963a; Cody et al., 1972; and Rakhawy et al., 1972). Collaterals from the mesencephalic neurons to the motor nucleus of the trigeminal nerve, possibly to the facial nucleus and to the cervical segments of the spinal cord and cerebellum have been described although the higher connections of the nucleus are not yet fully understood (Ramon y Cajal, 1909; Chan-Palay, 1977; Ruggiero et al., 1982; Rokx et al., 1986a).



1.48 Meynert (1872) associated the mesencephalic root with the trigeminal nerve and noted its sensory characteristic. Ramon y Cajal (1896, 1909) subsequently studied the mesencephalic nucleus in man using staining methods and described a thick axon and a fine collateral passing to the motor nucleus of the trigeminal nerve and called it the accessory trigeminal motor nucleus. The early workers studied the mesencephalic nucleus using staining and degeneration methods to show the microscopic organisation of the nucleus, its distribution and neuron features in man and other animals such as <sup>the</sup> cat, rabbit and monkey (Johnston, 1909; Van Valkenberg, 1909; Weinberg, 1928).

1.49 In their major study, May and Horsley (1910) studied the mesencephalic nucleus using Marchi stain together with chromatolysis of cells following lesions of trigeminal nerve as well as intracerebral division of mesencephalic root in cats and monkeys. They found that the nucleus contains central and peripheral fibres that leave the pons by the motor root of the trigeminal nerve and which are distributed with some peripheral branches to the muscles of mastication. Willems (1911) first spoke of the proprioceptive nature of the mesencephalic nucleus for the muscles of mastication following his findings on avulsion of motor root branches of the mandibular division in the rabbit, which resulted in chromatolysis of nearly all the cells of the motor



nucleus of the trigeminal nerve and of about 51% of the mesencephalic nucleus. Studies of Kosaka (1912) in <sup>the</sup> dog and monkey and Allen (1919) in <sup>the</sup> cat and guinea pig further substantiated that the peripheral distribution of the mesencephalic nucleus to the muscles of mastication subserves proprioceptive function. Kosaka (1912) and Thelander (1924) further demonstrated that although the main mesencephalic nucleus branches leave mostly with the motor root, a few fibres enter the sensory branches of the trigeminal nerve.

1.50 The early studies on quantitative and qualitative aspects of the mesencephalic nucleus were conducted by Clark (1926); Schneider (1928); Weinberg (1928) and Sheinin (1930, 1933). These studies showed that the mesencephalic nucleus consists of predominantly large medium and small unipolar neurons, with a centrally placed nucleus and a distinct nucleolus, granular cytoplasm with Nissl granules. The histological observations of the mesencephalic neurons are characterised by their position in the periphery of the aqueductal gray matter and staining intensity. The neurons also show features of cell clustering of large and/or small neurons. The resemblance of the cells of the mesencephalic nucleus to the unipolar primary sensory neurons of cranio-spinal ganglia was noted by Allen (1919); Clark (1926); Weinberg (1928); Schneider (1928) and Sheinin (1930). Clark (1926) pointed out the close

similarity between the large cells of the mesencephalic nucleus and those of spinal ganglia which Warrington and Griffin (1904) demonstrated to be connected with muscle spindles.

1.51 The comparative study of the mesencephalic nucleus conducted by Weinberg (1928) in a number of vertebrates including primates revealed the distribution of the neuron cell bodies of the nucleus and confirmed the proprioceptive functional correlation to muscles of mastication. Schneider (1928) also made total cell counts in the mesencephalic nucleus of some mammals. Studies carried out by Freeman (1925) agreed with Willems (1911) in that mesencephalic root supplied proprioceptive fibres to the muscles of mastication as well as proprioceptive fibres to the extraocular muscles in the cat. Sheinin (1930) and Tarkhan (1934) found that in the rabbit and in the cat, the proprioceptive fibres to the extraocular muscles were from the cells of the mesencephalic nucleus.

1.52 Several studies have been conducted with the aim of obtaining total cell counts of the mesencephalic nucleus in a number of animal species. In the cat, the distribution and cell counts was carried out by Valkenberg (1909); Weinberg (1928); Tarkhan and Gabrawi (1967) Dault and Smith (1969); Hinrichsen and Larramendi (1969); Sivanandasingham and Warwick (1976). The

findings in the cat have revealed a total cell count of 400-693 (Valkenberg, 1909); 940-2400 (Dault and Smith, 1969); and 500-600 (Sivanandasingham and Warwick, 1976). Hinrichsen and Larramendi (1969) and Weinberg (1928) found as many as 2000-3000 cells and Capra et al. (1985), and Nomura et al. (1985), found about 1000 cells in the cat. In the dog, Kosaka (1912) and Sheinin (1930) found about 1100-2800 cells. In the mouse, Hinrichsen and Larramendi (1969) found 1434 cells in the adult mouse and 1552 cells in the new born mouse. The total cell counts in the rat were also found to be variable. Weinberg (1928) reported 2980 cells and Hinrichsen and Larramendi (1969) found 2434 cells, Rakhaw et al. (1972), found 1127-1910 cells. Foster (1973) and Sivanandasingham and Warwick (1976) found 578 and 748 cells respectively. Rokx et al. (1986a), found 1000-1600 cells in the rat. Cell counts in the mesencephalic nucleus of the rabbit, guinea pig and mole have been made by Valkenberg (1909) and Willems (1911). Valkenberg (1909) found 512 cells and Willems (1911) found 1501-1655 cells in the rabbit. Weinberg (1928) counted 434 cells in the mesencephalic nucleus of the frog and 537 cells in that of the turtle. Vegetti and Palmaeri (1965) found 900 neurons in the reptile while Luiten (1979), found 36-50 cells in the fish.

1.53 Studies on the structural organisation and cell counts in the non-human primates are limited and show

conflicting findings. Kosaka (1912) found 2744 cells and Weinberg (1928) found 4869 cells in one guenon monkey. Sivanandasingham and Warwick (1976) found 1132 cells, (corrected numbers were 698 and 862) in the 2 rhesus monkeys and 644-696 (corrected numbers were 467 and 498) in the mesencephalic nucleus of 2 slow-loris. They described 50% of the cells in mesencephalic nucleus to be large and 50% to be small in both primates. In the human, Weinberg (1928) found 5737 cells in one adult, while Valkenberg (1909) found 716 large and 25 small cells in one infant of 5 months.

1.54 It is noted, however, that the variation in the number of cell counts in the same species as observed by different authors may be due to the differences in serial sectioning, where there is the possibility of counting split cells, and the difference may also be seen if the counting is unilateral or bilateral (Abercrombie 1946, Konigsmark, 1970). Furthermore, there is the possibility of including cells of the oculomotor and trochlear nuclei as the neurons are proximal to the mesencephalic nucleus in the mid-brain. Also in the pons some sensory neurons of the trigeminal nucleus may be included. The difficulty of distinguishing cells of the locus coeruleus in the caudal portion of the mesencephalic nucleus in the region of <sup>the</sup> floor of the fourth ventricle may also account for a source of error in neuron estimation (Sheinin 1930; Weinberg, 1928; Hinrichsen and Larramendi, 1968, 1969;

Hubbard and Dicarlo, 1973; Sivanandasingham and Warwick, 1976).

1.55 In the rat, cat, mouse and monkey the mesencephalic nucleus is believed to extend as a crescent shaped band from the level of the superior colliculi in the midbrain to the floor of the fourth ventricle in the pons upto the level of the motor nucleus of the trigeminal nerve. The mesencephalic cells are found on the dorsal side of the periaqueductal gray matter in the region of the superior colliculi and then tend to be laterally placed at the level of the inferior colliculi. In the pons, the mesencephalic cells are found in the ventrolateral aspect of the periphery of the gray matter at the floor of the fourth ventricle. The number of cells found along the extent of the nucleus as described by Hinrichsen and Larramendi (1969) and Capra et al., (1985), in the cat and mouse shows some regions of higher cell density in the midbrain and pons. In the dog, Sheinin (1930) showed a higher cell concentration in the midbrain than in the pons. In the rat, Hinrichsen and Larramendi (1969) and and Rokx et al. (1986a), showed higher concentration of cells in the pons. In the monkeys, the cell distribution, position and concentration is similar to that in the cat (Sivanandasingham and Warwick, 1976).

1.56 The association of <sup>the</sup> mesencephalic nucleus to stimuli from the teeth and muscles of mastication was shown by Corbin (1940) who studied the peripheral distribution of mesencephalic fibres in the cat using degeneration method. The mesencephalic nucleus and root were destroyed and the various cranial nerves studied for degeneration by the direct osmic acid technique and Marchi technique. The peripheral distribution was found to be by the medium to large sized myelinated fibres to the ethmoidal branch of ophthalmic division, palatine and superior alveolar branches of <sup>the</sup> maxillary and into the pterygoid, masseteric and inferior alveolar branches of <sup>the</sup> mandibular nerve. These accounted for about 10-15% of the large myelinated fibres. There was no contralateral degeneration of <sup>the</sup> branches. Corbin (1940) showed that the mesencephalic root fibres, in addition to the muscles of mastication pass to the superior alveolar, palatine and inferior alveolar nerves, and suggested that these <sup>fibers</sup> supply deep sensations to the teeth, gums and palate, such that together with the fibres of muscles of mastication, served to control the force of the bite and thus prevent serious damage to the teeth gums and palate as well as reflexly controlling mastication. Corbin (1940) failed, however, to demonstrate any mesencephalic fibres in VIth, VIIth, IXth, Xth, XIth or XIIth cranial nerves. Whether the degeneration <sup>of</sup> fibres in the IIIrd and IVth cranial nerves were due to the injury to the motor nuclei of these

nerves during mesencephalic nucleus lesion was not clear but seems probable.

1.57 Corbin and Harrison (1940) evaluated the function of the mesencephalic root of the fifth cranial nerve in the cat using electrophysiological method of recording action potentials. They recorded action potentials, characteristic of proprioceptive impulses elsewhere, from all portions of the mesencephalic root in response to jaw opening and thus to stretching of muscle of mastication. The recordings were ipsilateral. The mesencephalic neurons receiving input from the muscles of mastication have a rostro-caudal distribution in the mesencephalic nucleus. Action potentials were elicited in the caudal half of the nucleus from blunt pressure stimulation of the ipsilateral teeth and hard palate. In the cat, the canine teeth were the most responsive of the oral structures. The physiological evidence was thus functionally correlated to the anatomical findings of Corbin (1940). The study of Imamoto (1972) showed that there was collateral connection from the mesencephalic nucleus to the motor nucleus of <sup>the</sup> trigeminal nerve.

1.58 Impulses passing in the alveolar and palatine nerves to the mesencephalic nucleus are probably chiefly inhibitory, preventing damage to the gums, teeth and palate. The dental and palatal impulses as well as those from the muscles of mastication, mediated by the





mesencephalic root fibres constitute the afferent limb of the masticatory reflex arcs, thereby coordinating and controlling mastication (Sherrington, 1917; Szentagothai, 1948). No action potentials were elicited from the mesencephalic nucleus as a result of stretching the extraocular muscles, so their association with this nucleus is doubtful. Corbin (1940) and Corbin and Harrison (1940) clarified the function and peripheral distribution of the fibres from mesencephalic nucleus and showed that the nucleus is activated by jaw opening movements and pressure stimulation of teeth, gums and palate. They also showed that the jaw-jerk reflex is abolished by lesions of the nucleus (Harrison and Corbin, 1942).

1.59 Jerge (1963a) using electrophysiological method, carried out a study of the topographical organisation and function of the mesencephalic nucleus in the cat. He found three types of neurons. There were 84 cell units (78%) innervating muscle spindles of masseter, temporalis and medial pterygoid muscles. No units were observed in association with lateral pterygoid muscles. There were two types of neurons innervating dental pressoreceptors. There were 13 Type 1 pressoreceptor neurons which responded to stimulation of single tooth. The response was regardless of direction of pressure applied. There were 4 rapidly adapting and 9 slowly adapting units. Of the 13 Type 1 units (12%), 6 were elicited from



stimulation of maxillary canine, 5 from mandibular canine, one from maxillary molar and one from mandibular third premolar. The Type II dental pressoreceptor neurons innervated two or more adjacent teeth and in some cases contiguous gingival areas. The Type II neurons had more complex peripheral fields and presumably had branching axons to do so. There were 11 (10%) Type II units. 7 units elicited response from pressure on teeth; one from (Max I<sup>3</sup>, C, PM<sup>I</sup> and M<sup>I</sup>), five from (Max C, PM<sup>I</sup>, M<sup>I</sup>), one from mandibular (I<sub>3</sub>, C) and 4 units elicited response from the surrounding soft tissue as well. Jerge (1963a), found the muscle spindle neurons along the rostro-caudal extent of the nucleus. Type I dental pressoreceptor neurons were localized within 2mm on either side of the Horseley-Clark Zero coronal plane and Type II dental pressoreceptor neurons were localized in the caudal half of <sup>the</sup> mesencephalic nucleus. There was no apparent pattern in the distribution of the units in either dorsoventral or mediolateral extent of the nucleus. The maximum threshold forces required for the Type I pressoreceptor units were in the range of 1-3g with mean of 1.8g and for the Type II units, higher thresholds of 2-6g were required along the axis of maximum sensitivity. All the mesencephalic neurons were activated solely from homolateral (ipsilateral) fields. A study of the latency for units related to <sup>the</sup> dental pressoreceptors showed considerable variation in the

latency and findings agree with histological evidence establishing the first order nature of the mesencephalic neurons. In general, findings of Jerge (1963a) agree with the work of Corbin (1940); Corbin and Harrison (1940) and Szentagothai (1948).

1.60 Dault and Smith (1969) also provided data in the cat using degeneration method, of chromatolytic neurons following nerve section. They found bilateral representation in <sup>the</sup> mesencephalic nucleus, of masseter, temporalis, inferior alveolar, lingual and hypoglossal nerves. Cody et al. (1972), in their study of the functional analysis of the mesencephalic nucleus in the cat using extracellular microelectrode recordings confirmed the findings of Corbin and Harrison (1940) and Jerge (1963a). They found two types of units, the muscles spindle first order afferents of ipsilateral jaw closing muscles responding to muscles stretching and mechanoreceptor afferents of ipsilateral maxillary and mandibular teeth responding to pressure on <sup>the</sup> teeth. The muscle spindle units were distributed in all parts of the mesencephalic nucleus. No evidence for representation of extraocular muscle receptors was found. The cell units responding to pressure on teeth were most commonly associated with the canine teeth. Some of the units were quite specific with regard to direction of pressure on a single tooth, while others could be excited by pressure on several teeth or surrounding gum, supporting Jerge's

(1963a) work. It is thus fairly certain that cells recorded in the mesencephalic nucleus can only belong to jaw-closing muscle spindles or tooth receptors. Cody et al. (1974), in a further study in the cat considered the distribution of tooth receptor afferents in the mesencephalic nucleus. Of the 361 units examined in 14 cats, 47 (13%) belonged to tooth receptors with properties similar to those described by Jerge (1963a). It was also found that <sup>the</sup> tooth receptor afferents were concentrated in the caudal part of the midbrain. (Cody et al., 1974). Jerge (1963a) had found some dental units in the rostral part which Cody et al. (1974), suggested may have been due to the difficulty of localization when penetrating through the whole cortex at an angle of  $30^{\circ}$  to the vertical. Cody et al. (1974), further suggested that the caudal distribution may have some significance in relation to the electrotonic coupling observed by Baker and Llinas (1971) between cells of the caudal part of the mesencephalic nucleus.

1.61 Linden (1978) evaluated the properties of intraoral mechanoreceptors represented in the mesencephalic nucleus of the cat using extracellular electrodes. He confirmed that the primary afferent intraoral mechanoreceptor fibres have their cell bodies in this nucleus. Two groups of intraoral mechanoreceptors were found. Linden (1978) recorded 325 intraoral mechanoreceptor neurons which gave a single all

or none response to a single electrical stimulus applied either to <sup>the</sup> superior dental, inferior dental or the palatine nerve and all were able to follow stimulus frequency of over 100 HZ for over 2 sec. The action potentials evoked by mechanical stimulation of the tooth were similar to those evoked by stimulation of the peripheral nerve. 285 of the intraoral mechanoreceptor neurons were of the first group, consistent with the periodontal mechanoreceptor neurons described in the earlier studies (Jerge, 1963a, Cody et al., 1972). The response characteristic differed in two respects from the earlier studies in that there were no neurons that responded for over 10 seconds to a sustained application of a supra-threshold mechanical stimulus to the teeth and there were no spontaneously active neurons of the first group. Of the 285 neurons, Linden (1978) found 260 of these responded to the left mandibular teeth and the inferior alveolar nerve while 25 responded to the forces on left maxillary teeth and the stimulation of left superior dental nerve. On a further analysis of the 260 mandibular periodontal mechanoreceptor neurons, 153 responded to forces applied to canine, 53 to molar, 39 to second premolar, 8 to first premolar, 3 to third incisor, 3 to second incisor and 1 to first incisor.

1.62 On considering the physiological properties of mechanoreceptor neurons, Linden (1978) found 15% of the mechanoreceptor neurons to be rapidly adapting in that

they responded while <sup>the</sup> force was being applied but did not continue to fire to a sustained force. The other 85% were slowly adapting i.e. they responded to both <sup>the</sup> phasic and sustained components of force. No spontaneously discharging neurons were found. All the mechanoreceptor neurons exhibited directional sensitivity, in that, they responded maximally to a force on the tooth in one particular direction. Further, the receptive fields for the maxillary and mandibular neurons were confined to one tooth, no neuron responded to mechanical stimulation of gingivae only or both tooth and gingivae.

1.63 The second group of intraoral mechanoreceptor neurons responded to electrical stimulation of the ipsilateral palatine nerve and responded to forces applied to all the maxillary teeth, both contralateral as well as ipsilateral and also to forces applied to nose and hard palate. Linden (1978) termed the second group of neurons as Type P. 40 Type P neurons were found. These were slowly adapting, also showing direction sensitivity, their characteristic being that they produced greater discharge when a force equivalent to IN was applied to the ipsilateral teeth than when the same force was applied to the contralateral teeth. The actual site of the Type P neuron receptors is, however, unknown. Goodwin and Luschei (1975) mentioned that some maxillary mechanoreceptor neurons had receptive fields which extended over the whole maxillary arch. Corbin (1940) had

earlier shown that the degeneration of <sup>the</sup> palatine nerve fibres occurs after placing lesions in the mesencephalic nucleus and tract which suggests that there are, in the nucleus, some cell bodies of <sup>the</sup> primary afferent fibres running in the palatine nerve in conformity with electrophysiological findings (Linden, 1978). The greatest difference between Type P and periodontal mechanoreceptor neurons is the receptive fields. Anatomically, it is unlikely that the palatine nerve provides branches to <sup>the</sup> receptors around the maxillary teeth on both sides. Rather it is suggested that the receptors may be situated in the sutural tissue (Sakada 1974, Sakada and Okomoto, 1975) and that a force applied to any maxillary or supramaxillary structure such as the nose is transmitted through the bone to these receptors. The possible site of the receptors may be in the palatomaxillary suture as the forces extended on a canine are distributed across the fibrous palatomaxillary suture and not along the midline sutures (Buckland-Wright, 1978). It is not clear however, whether the receptors respond either to compression or tension of the surrounding tissue.

1.64 According to Linden (1978) the recording sites of both periodontal and Type P neurons are situated in the caudal part of the mesencephalic nucleus. Though Cody et al. (1974) had suggested that the caudal position of these neurons may have some significance in relation to

possible electrotonic coupling between cells of the caudal part of <sup>the</sup> nucleus, there is little evidence from the electrotonic studies for coupling to be predominant in the caudal part of the nucleus (Hinrichsen, 1970; Baker and Llinas, 1971). Linden (1978) discussed that if there was electrotonic coupling between muscle-spindle and intraoral mechanoreceptor neuron, one would expect to record from single neurons that respond to both opening of the mouth and mechanical stimulation of the teeth. Linden (1978) found none of the mechanoreceptor neurons to respond to both forms of stimuli, jaw movements and mechanical stimulation of teeth, and suggested that if coupling does exist between cells in the mesencephalic nucleus of the cat, this could not be between intraoral mechanoreceptor and muscle-spindle.

1.65 The receptive fields for the periodontal mechanoreceptor neurons found by Linden (1978) were similar to the single tooth units described in peripheral studies (Sakada and Kamio, 1971). Jerge (1963a) found that 19 out of 24 units responded to forces applied to the canines. Corbin and Harrison (1940) noted a bigger response from <sup>the</sup> mesencephalic nucleus when pressure was applied to the canines than to any other oral structure. Linden (1978) found that the canine had the largest representation in the mesencephalic nucleus, over 50% of those in the inferior alveolar nerve. Kruger and Michael (1962) observed that the majority of cells in the main



sensory and spinal nuclei also responded to forces applied to the canines. Mei et al., (1975), found most of the periodontal mechanoreceptors to be located in the canine teeth in the cat. The functional significance of large canine representation is not yet apparent.

1.66 Passatore et al. (1983), studied the localization of the neurons innervating<sup>the</sup> masticatory muscle spindles and<sup>the</sup> periodontal receptors in the mesencephalic nucleus of trigeminal nerve of the rabbit using electrophysiological methods. They found 210 units which supplied periodontal mechanoreceptors and 396 which were innervating jaw elevator muscle spindles. The two groups of neurons were segregated within the nucleus, the caudal portion containing mostly periodontal neurons. Of the periodontal neurons, in the rabbit, incisors showed the highest representation of 40%, interalveolar gingivae 29% and molars 12%. The remaining units had wide receptive fields similar to Type II units of Jerge (1963a). 75% of the periodontal units were slowly adapting while 25% were rapidly adapting and showed directional sensitivity as found by Linden (1978). The threshold ranged from 0.5 and 50g, being much lower for incisors than for molars. <sup>The</sup> mesencephalic cells were found to respond to mechanical stimuli given on contralateral teeth or gingival areas. Passatore et al. (1983), also sectioned the inferior alveolar nerve on one side and found chromatolytic neurons in both ipsilateral and contralateral



mesencephalic neurons, predominantly in the caudal part of the nucleus.

1.67 The anatomical neuronal tracing methods have supplemented and confirmed most of the electrophysiological findings of the peripheral distribution of the mesencephalic nucleus. Uptake of HRP was demonstrated from <sup>the</sup> muscles of mastication to the motor nucleus and <sup>the</sup> mesencephalic nucleus of trigeminal nerve (Alvarado-Mallart et al., 1975). Injection of HRP into the temporalis, masseter and medial pterygoid <sup>muscles</sup> in the rat and cat showed topographic location of <sup>the</sup> motor neurons supplying the jaw-elevator muscles as well as the mesencephalic neurons innervating the muscle spindles (Mizuno et al., 1975). Recently, Jacquin et al. (1983b), has shown the topographic location of <sup>the</sup> motor neurons and somatotopic organisation of <sup>the</sup> mesencephalic neurons innervating the jaw-elevator muscles and teeth in the rat using retrograde HRP tracing method. The mesencephalic neurons innervating the muscles were located along the rostro-caudal extent of the nucleus. The mesencephalic neurons were unipolar with unimodal distribution of major and minor axis which ranged from 12-42 $\mu$ m and 5-25 $\mu$ m respectively. The mesencephalic neurons innervating teeth, which have the peripheral processes in the inferior alveolar nerve and infraorbital nerve were located mainly in the caudal part of the mesencephalic nucleus. In addition, application of HRP to <sup>the</sup> inferior alveolar and

mandibular nerve labelled cells in the supratrigeminal nucleus.

1.68 The morphology, location and morphometric analysis of the mesencephalic neurons innervating the masticatory muscles of the cat have been studied using HRP method. Walberg (1984) and Nomura et al. (1985), found not only HRP labelled large and small unipolar cells but also multipolar cells in the ipsilateral mesencephalic nucleus following HRP injection in muscles of mastication. The multipolar cells were found mainly in the pontine part and were faintly labelled compared to the unipolar cells. Capra et al. (1985), used computer image analysis to measure the mesencephalic neurons. <sup>The</sup> large and small unipolar cells and multipolar cells innervating the masseter and temporalis muscles were identified throughout the rostro-caudal extent of the nucleus. No clear somatotopy or segregation of <sup>the</sup> muscle spindle afferent neurons of these muscles was noted.

1.69 Retrograde HRP labelling of primary neurons in the caudal part of ipsilateral mesencephalic nucleus and in the supratrigeminal nucleus was demonstrated by application of HRP to <sup>the</sup> maxillary nerve by Gonzalo-Sanz and Insuasti (1980). These neurons were interpreted as being responsible for sensitive innervation of the periodontal ligament. Matesz (1981) used the method of cobalt labelling technique in the rat and showed

mesencephalic afferent fibres in all three divisions of the trigeminal nerve. Collaterals of the mesencephalic neurons were shown terminating mainly in the supratrigeminal nucleus and the motor nucleus of the trigeminal nerve. The mandibular nerve application of cobalt chloride filled the mesencephalic neurons rostro-caudally while the ophthalmic and maxillary nerve labelled cells only caudally. Marfurt (1981b) used HRP method to show the central connections of some of the branches of trigeminal nerve. He found HRP labelled brainstem sensory neurons following HRP application to the trigeminal nerve branches. He also showed mesencephalic labelled neurons following HRP application to the inferior alveolar nerves. He further showed that there was direct collateral connection of periodontal mesencephalic afferent neuron to the cerebellum while there was no direct collateral connection of the periodontal mesencephalic afferent neuron with the jaw-opening and jaw-closing motoneurons (Szentagothai, 1948; Imamoto, 1972; Hannam, 1976).

1.70 Gottlieb et al., (1982), and Boseley et al., (1983), demonstrated the distribution of HRP labelled periodontal receptor afferents in the mesencephalic nucleus of the cat to be in the caudal part of the ipsilateral mesencephalic nucleus. In a subsequent

study, Gottlieb et al., (1984), found mesencephalic labelled neurons belonging to the proprioceptor afferents of jaw closing muscles distributed throughout the full extent of the nucleus. When HRP was applied to the inferior alveolar nerve, infraorbital nerve, and periodontal ligament, labelled cells were found in the ipsilateral trigeminal ganglion and mesencephalic nucleus. The labelled mesencephalic cells were identified as belonging to the periodontal receptor afferents and were restricted to the caudal region of the nucleus. The findings of Gottlieb et al., (1984), it seems, form one of the first attempts to demonstrate the periodontal ligament afferent neurons in the mesencephalic nucleus using the anatomical method of retrograde HRP labelling. The authors noted that although HRP was retrogradely transported when injected into the intact periodontal ligament around the teeth and into several sites in the masseter muscle, it was considered that uptake of HRP by muscle spindles and periodontal receptors was unlikely to have been complete because of their connective tissue capsule and spatial distribution of the receptors. In subsequent experiments HRP was applied directly to cut nerves such as inferior alveolar (innervating mandibular teeth), infraorbital (superior alveolar branch innervating maxillary teeth) and the masseteric nerve. When HRP was applied to the

inferior alveolar nerve at the ramus; 7.3% of ipsilateral mesencephalic neurons were labelled as compared to 5.9% when the HRP was injected into the periodontal ligament of the teeth. When HRP was applied at the mandibular foramen, the "whole" inferior alveolar nerve labelled 20% of the mesencephalic neurons. The infraorbital nerve application of HRP labelled 16.8% of the mesencephalic neurons. When considering the "best" counts of the whole inferior alveolar and infraorbital nerves representing the periodontal afferents of <sup>the</sup> maxillary and mandibular teeth, the number of labelled neurons was 46.9% of the total population, found mainly in the caudal part of the nucleus. Nomura et al., (1985), have applied HRP to trigeminal nerve branches in the cat and found that about 30% of the mesencephalic neurons innervate teeth

1.71 The anatomical findings of Gottlieb et al. (1984), confirm and extend the electrophysiological findings of Cody et al., (1974), and Linden, (1978), in that the periodontal afferent neurons are ipsilateral and concentrated in the caudal part of the nucleus in the cat. Linden (1978) found 260 units responding to the left mandibular teeth of which 153 responded to canine, 53 to molar, 47 to premolars and 7 to incisors. Only 25 units responded to maxillary teeth. Gottlieb et al. (1984), findings suggest that maxillary teeth periodontal

afferent neurons are about 16.8% compared to 20% of the mandibular and that the anterior mandibular teeth afferent neurons are about 7.3% compared to about 12% of posterior teeth.

1.72 Chiego et al., (1979), and Byers et al., (1986), in their study in the cat, on anterograde axoplasmic transport of  $H^3$  leucine from the mesencephalic nucleus found discrete labelling of nerve endings at the apical one third of the periodontal ligament as well as close to the mucosa of the hard palate. No contralateral labelling was seen supporting the studies of Corbin and Harrison (1940) and Cody et al., (1972).

1.73 Attempts have been made to elucidate the innervation of dental pulp by <sup>the</sup> mesencephalic neurons but the information available is relatively poor and conflicting. Chiego et al., (1979), found sparse pulpal labelling of <sup>the</sup> mesencephalic terminals in the cat while Byers et al., 1986, did not observe labelled mesencephalic axons in the pulp in the cat. In their study in the primate, Chiego et al., (1980), found  $H^3$  HRP labelled mesencephalic cells following the application of the tracer in the dental pulp supporting earlier findings of Cox et al., (1977). However, Marfurt and Turner (1984), and Capra et al., (1985) failed to demonstrate retrogradely labelled mesencephalic cells

following HRP injection into the tooth pulp in the rat and cat. Much remains to be known about whether or not the conflicting results are due to the leakage of the tracer through the pulp into the apical periodontium. Using two axonal tracers, one for tooth pulp (HRP) and <sup>the</sup> other for periodontal ligament (DAPI) Capra et al., (1984) have demonstrated in the cat mandibular canine, the neuronal somata that innervate tooth pulp and adjacent periodontal tissues. HRP and DAPI labelled cells neurons were seen in the ipsilateral mandibular part of trigeminal ganglion and only DAPI labelled were located in the mesencephalic nucleus. The study further showed that there was no collateralization of <sup>the</sup> single neurons to innervate both, pulp and periodontal ligament.

1.74 There is conflicting evidence on whether the mesencephalic root projects to the ipsilateral as well as the contralateral nucleus. Physiological and anatomical studies in the cat by Smith et al., (1967), Dault and Smith (1969); <sup>and</sup> Rakhawy et al., (1972); have indicated that in addition to <sup>the</sup> masseter and temporalis afferents passing to the ipsilateral nucleus, some may project to the contralateral nucleus. Rogers and Cowan (1973) found similar evidence in chick and Rakhawy et al., (1972) in the rat in their histological studies.

1.75 As chromatolytic cells are difficult to distinguish from degenerative cells, doubt has been expressed of a bilateral projection to the mesencephalic nucleus. HRP studies of Hinrichsen (1976) failed to label contralateral mesencephalic neurons following injection of HRP into <sup>the</sup> masseter muscle. Physiological evidence has been lacking (Corbin and Harrison, 1940; Jerge, 1963a) to support the earlier findings of Smith <sup>et al</sup> (1967), although Passatore et al., (1973), in the rabbit, found some evidence of bilateral connection. Desole et al., (1970) found no responses in the contralateral nucleus to stretch of masseter muscle in reptiles and Nakamura et al., (1973a,b), found none in <sup>the</sup> cat. Gottlieb et al., (1984), found no contralateral connection to mesencephalic nucleus in the cat from muscles as well as teeth. Capra et al., (1984, 1985), Rokx et al., (1986a), Byers et al., (1986), have demonstrated only ipsilateral afferent neurons in the mesencephalic nucleus of muscle spindles and periodontal mechanoreceptors in the cat and rat. Ipsilateral connections from muscles to the mesencephalic nucleus have been found in the monkey and rat. (Ibrahim and Leong, 1979; Jacquin et al., 1983a).

1.76 From the foregoing review, the electrophysiological and anatomical studies in the cat,



rat and rabbit show that the periodontal ligament mechanoreceptor afferents neurons are found mainly in the caudal part of the mesencephalic nucleus at the level of the inferior colliculi and the floor of the fourth ventricle in pons (Corbin and Harrison, 1940; Jerge, 1963a; Cody et al., 1972, 1974; Linden, 1978; Passatore et al., 1983; Gottlieb et al., 1984; Capra et al., 1984). Studies of Jerge (1963a); Cody et al., (1972), and Linden (1978) show that canine in the cat has a large representation in the mesencephalic nucleus. In the rabbit, Passatore et al., (1963), found that incisors were most widely represented. The majority of the studies indicate that the periodontal afferent connections of the mesencephalic nucleus are ipsilateral in the cat (Cody et al., 1972; Gottlieb et al., 1984), while Passatore et al., (1983), found some contralateral projecting afferents in the rabbit. Apart from the study of Chiego et al., (1979, 1980), in the cat and in the monkey, mesencephalic afferents to the dental pulp have not been shown in the rat (Marfurt and Turner, 1984) and in the cat (Capra et al., 1984; Byers et al., 1986).

1.77 There is experimental evidence to show that the neurons comprising the mesencephalic nucleus migrate into the brain from the neural crest and the developmental history in association with the anatomy and physiology of

the mesencephalic cells makes it reasonable to consider the nucleus to be a homologue of the craniospinal sensory ganglia (Weston, 1970; Narayan and Narayan, 1978). Development of the human mesencephalic trigeminal nerve was studied by Windle and Fitzgerald (1942) who found that the mesencephalic root has its genesis in a lateral longitudinal fascicle in common with other descending fibres. The mesencephalic nucleus differentiates in a caudo-rostral direction. Pearson (1949), in the study of mesencephalic nucleus in human embryos, fetuses, newborn and adult found that the neurons differentiate in the outer part of the central gray matter of the outer plate of the mesencephalon. The processes of the cells form a fibre layer at the outer boundary of the central gray matter. Lewis and Straznicky (1979) studied the time of origin of the mesencephalic neurons in xenopus with  $H^3$  thymidine autoradiography and reported that the generation time of the mesencephalic cells and trigeminal ganglion cells were dissimilar suggesting, therefore that the cells do not have a common lineage and that mesencephalic cells are not from the neural crest. They propose that mesencephalic cells are derived through a proliferative activity of the precursor cells in the mesencephalic junction and tectum neuroepithelium in the xenopus. The dual embryonic origin of the nucleus from

neural crest and ectodermal plate might account for the non-homogeneity of this nucleus (Dubner et al., 1978).

1.78 At the electron microscopic level, the mesencephalic neurons show an initial axon segment similar to that of sensory ganglion cells (Hinrichsen and Larramendi, 1970; Alley, 1973). The initial axon segment is free of synaptic contacts and without a subaxolemmal undercoating or fasciculated microtubules. The axon hillock region also resembles that of sensory ganglion cells in being almost Nissl-free (Brodal and Saugstad (1965) and at the ultrastructural level containing few ribosomes (Alley, 1973).

1.79 However, in spite of the structural, functional and embryological affinities between the mesencephalic neurons and sensory ganglion cells, there are some unique differences. The most fundamental difference is that, unlike the sensory ganglion cell bodies which are completely ensheathed by satellite cells and devoid of synaptic contacts, mesencephalic neurons are set in an area of complex neuropil (Imamoto and Shimizu, 1970) and are only partially enclosed by glial cell processes so that somal surface comes into direct contact with neuropil elements, particularly with sparse axon terminals that establish axosomatic synapses (Hinrichsen and Larramendi, 1968, 1970; Imamoto and Shimizu, 1970;

Lucchi et al., 1972; Bortalami et al., 1972; Alley, 1973, Nomura et al., 1985). Not much is known about the physiological significance or sources of these axo-somatic endings.

1.80 Whether the variations in the many histological descriptions of cell morphology, type and distribution within the mesencephalic nucleus reflect functional, phylogenetic and/or ontogenetic differences are still uncertain (Dubner et al., 1978). Ramon y Cajal (1909) considered that neurons change from multipolar to unipolar cells and are more common in the lower animals. Cell numbers decreased markedly in the perinatal period (Rogers and Cowan, 1973; Alley, 1974) and the number of spines and synaptic contacts may increase in number after birth which may partly account for ontogenetic factors. In the morphological study of mouse mesencephalic nucleus, the newborn mouse had one to five dendritic processes on the soma. In the 6 day old mouse, there were no distinct multipolar cells but there were small spines on the soma of most cells, which were no longer present in the adult mouse (Hinrichsen and Larramendi, 1969). Alley (1974) suggested that the spinous processes served a nutritive function rather than receiving synapses.

1.81 The mesencephalic neurons give rise to numerous short spines and crests (Ramon y Cajal, 1909). Imamoto and Shimizu (1970) found rare synaptic contacts on such spines, while Hinrichsen and Larramendi (1970) and Alley (1973) found the spines devoid of synaptic contacts. The functional significance of these synaptic contacts, of which there may be two types and which become evident after birth and increase in density as the animal matures (Alley, 1973) remains uncertain. The spines are probably paraphytes rather than the usual postsynaptic spines of neurons in the central nervous system. The site of origin of the synapses in terms of presynaptic inputs and whether the synapses are inhibitory or excitatory is not known. (Nomura et al., 1985). The presence of synapses on the cell bodies or primary afferents suggest that the inflow of sensory information from jaw muscle proprioceptors and periodontal mechanoreceptors may be subject to presynaptic control (Miles, 1979). The sensitivity of some other sensory systems is known to be subject to centrifugal control. The best examples are the muscle spindle (Matthews, 1964), the organ of Corti and vestibular organ (Klinke and Galley, 1974) although efferent control in these cases is extended at the receptor level. Thus, it is possible that jaw muscle proprioceptors are subject to control both at the

receptor (by means of fusimotor fibres) and at the sensory nucleus.

1.82 The clustering of mesencephalic neurons into small groups of cells as observed by Ramon y Cajal (1909); Weinberg (1928); Hinrichsen and Larramendi (1969) and seen in vitro (Hild, 1957) represents another peculiar feature of the mesencephalic neurons. Ultrastructural studies have shown that extensive areas of direct plasmalemmal contact occur between neurons within such clusters, and that the interface is characterized by a series of closely spaced adherens-like maculae (Hinrichsen and Larramendi, 1968, 1970; Imamoto and Shimizu, 1970) and by areas of close membrane apposition resembling gap junctions (Hinrichsen and Larramendi, 1968, 1970; Lucchi et al., 1972). Passatore et. al., 1983. Evidence of electrotonic coupling between the neurons within a cluster (Baker and Llinas, 1971), suggests that the cells of a cluster may function as a unit, with ionic coupling between them mediated by gap junctions. Electrotonic coupling has also been shown in the inferior olive (Llinas et al., 1974); lateral vestibular nucleus (Korn et al., 1973); and abducens nucleus (Gogan et al., 1974). A consequence of coupling between neurons is the electrical synchronization of firing of the coupled cells. The function of the presynaptic endings on mesencephalic neurons is that they may regulate the strength of

coupling between electrotonically coupled neurons (Spira and Bennet, 1972).

1.83 Hinrichsen (1970) presented evidence suggesting that an electrotonic pathway may exist between some cells of the trigeminal mesencephalic nucleus and that the soma-somatic and axosomatic membrane fusions observed at the ultrastructural level may provide such pathways. Although Hinrichsen (1976) has estimated that mesencephalic cells may support as many as 100 synaptic contacts, synaptic activity in mesencephalic nucleus has not, as yet been demonstrated in mammals (Baker and Llinas, 1971). The functional significance of synchronization of firing in the proprioceptive afferents from the jaw muscles and/or periodontal mechanoreceptors is not clear. The significance of the caudal distribution of the intraoral mechanoreceptors within the mesencephalic nucleus (Corbin, 1940; Cody et al., 1974; Linden, 1978) has been suggested by Cody et al., (1974), to be in relation to possible electrotonic coupling between cells of the caudal part of the nucleus. However, there is no evidence from electrotonic studies (Hinrichsen, 1970; Baker and Llinas, 1971) for the coupling being predominantly in the caudal part of the nucleus. Hinrichsen (1970) and Baker and Llinas (1971) reported that fibres in the masseteric branch of the trigeminal nerve were involved, but did not show whether the coupling was between two muscle spindle cells or

muscle spindle and intraoral mechanoreceptor cells. If there was electrotonic coupling between muscle spindle and intraoral mechanoreceptor cells, one would record from single neurons that respond to both, opening of the mouth and mechanical stimulation of the teeth. Linden (1978) found none of <sup>the</sup> 325 intraoral mechanoreceptor neurons to respond to both jaw opening and tooth tapping, and concluded that if coupling does exist between cells in the mesencephalic nucleus of the cat, it is not between intraoral and muscle spindle cells.

1.84 The function of electrotonic coupling in the mesencephalic nucleus, if it exists, must depend upon the types of afferent neurons involved. Hinrichsen (1976) concluded that it occurred between spindle afferent somata, and thus would "amplify" the monosynaptic response to lengthening of jaw-closing muscles. Electrotonic coupling may exist in mesencephalic nucleus in selachian species between cells belonging to dental afferents and be involved in the rapid monosynaptic jaw snap reflex evoked by tapping the teeth (Roberts and Witkovsky, 1975).

1.85 Another striking difference between mesencephalic neurons and sensory ganglion cells is revealed by their different responses to axonal injury. The mesencephalic neurons of fully grown mammals, Cupedo (1970) and birds Bortolami et al., (1972), appear to be unusually



sensitive to lesions of their peripheral processes and undergo degeneration following lesions. Such lesions would not produce a severe response in sensory ganglion cells, subjected to interruption of their peripheral processes at comparable distance from the cell body in animals of similar age (Lieberman, 1976). The difference in reaction of sensory ganglion cell and a mesencephalic nucleus cell in response to interruption of the centrally directed process is more striking. The sensory ganglion cells show little histological or ultrastructural changes even when the injury is close to cell body while the mesencephalic cells show marked perikaryal changes following damage to their central axons (Brodal and Saugstad, 1965; Bortalami et al., 1972).

1.86 Apart from the motor nucleus of trigeminal nerve (Ramon y Cajal, 1909) collateral branches of large mesencephalic neurons have been traced to the hypoglossal nucleus and spinal cervical segments C1 and C2 (Szentagothai, 1948). In a study of the cat (Matsushita et al., 1981); using HRP method, labelled mesencephalic neurons were observed ipsilaterally when HRP was injected to spinal cervical cord at C1, C2 and C3. The extent of labelling was at the level of inferior colliculi, level of trochlear and oculomotor nuclei, but none at the level of superior colliculi. Thus, the mesencephalic nuclei project directly to the spinal cord (Corbin, 1942). Mizuno and Sauerland (1970) observed that the

descending fibres derived from lesions placed in caudal part of the mesencephalic nucleus pass in the dorsal aspect of the cord to terminate in the medial part of the ventral horn as far as C4. They confirmed the anatomical findings by electrophysiological studies. Sumino and Nzokai (1977) have shown that the neck motor neurons receive, in addition, input from lingual, masseteric and inferior alveolar nerve suggesting that the trigeminospinal reflex is induced not only by cutaneous input from <sup>the</sup> face, but also by input from intraoral structures. Szentagothai (1948) suggested that the spinal collaterals are part of an inhibitory pathway from jaw closing muscles to their infrahyoid antagonists. Matesz (1981) has shown extensive peripheral and central distribution of mesencephalic fibres in the cat.

1.87 Dacey (1982), in the study of axon morphology of the snake mesencephalic neurons using HRP iontophoretic extracellular injection method, showed that the mesencephalic cell axon can be divided into central, peripheral and descending branch. The central branch descends from the cell body in mid-brain to dorsal aspect of motor nucleus of trigeminal nerve and the motor root where it splits into peripheral and descending branches. The descending branch goes towards the spinal cord while the peripheral branch passes with the motor root of trigeminal to leave the brain-stem. All three branches have collaterals which distribute terminal swellings

the motor within nucleus of the trigeminal nerve. Single mesencephalic neurons diverge to contact a large number of motor neurons within the nucleus suggesting that single neurons receive a divergent input from motor neurons. The descending branch sends collaterals to the entire sensory nucleus of <sup>the</sup> trigeminal nerve. The overall pattern of mesencephalic axons resembles that described for spinal Ia afferent fibres in <sup>the</sup> cat.

1.88 Central projections of mesencephalic nucleus to brain-stem, spinal cord, cranial nerve nuclei, supratrigeminal zone and midbrain reticular formation have been described (Panneton, 1981; Herdman, 1980; Matsu<sup>shita</sup> et al., 1981; Rokx et al., 1986 a). Ruggiero et al., (1982), in the study of <sup>the</sup> rat and rabbit using HRP method reported that mesencephalic nucleus did not project to the medullary cranial nerve nuclei or spinal cord but to the several levels of parvocellular nucleus of medullary reticular formation.

1.89 Brodal and Saugstad (1965) reported a cerebellar projection from the mesencephalic nucleus which was also observed by Cupedo (1970), the fibres reaching the cerebellum by way of superior cerebellar peduncle. Collaterals to the cerebellum were also observed in the cat by Weinberg (1928), and Pearson (1949) and Marfurt, (1981b). Hinrichsen and Larramendi (1969) failed to demonstrate cerebellar projection from mesencephalic

nucleus in the mouse, and Robinson (1970) in <sup>the</sup> frog. However, Taylor and Elias (1984), Elias and Taylor (1984) and Elias et al., (1985); have shown evidence of direct cerebellar projection from mesencephalic nucleus suggesting a function of modulating sensory information. Species differences or different criteria and experimental difficulties may have contributed to this uncertainty though Chan-Palay (1977) has shown a substantial projection from the mesencephalic nucleus to the cerebellum using HRP method. Mesencephalic neuron connection to the few muscle spindles in the facial musculature are uncertain (Binns, 1974).

1.90 The role of cortical activity evoked by proprioceptive afferent stimulation has been viewed as sensory but subconscious feedback that is utilized by the cortex and other higher centres in the regulation of movements (Matthews, 1972; Passafiume et al., 1979). Lund and Sessle (1974) noted that cortical neurons controlling jaw closure receive an excitatory input from muscles and also from periodontal mechanoreceptors (Lund and Lamaire, 1973).

## 2. MATERIALS AND METHODS

### A. Animals

2.1 Ten vervet monkeys (*Cercopithecus aethiops pygerythrus*) and 13 olive baboons (*Papio anubis cynocephalis*) were used in the study for HRP retrograde tracing of neural connections of teeth. Of the 10 monkeys, three monkeys were from a pool of monkeys at the Institute of Primate Research of the National Museums of Kenya. These monkeys had been captured from the wild and were being used for bilharzia (*Schistosomiasis*) research project. Seven of the ten vervet monkeys were obtained from a licensed dealer in Nairobi specifically for the present study. Of the 13 baboons, seven were provided by the Wellcome Research Institute, Nairobi, while the remaining 6 baboons were provided by the Institute of Primate Research. The two groups of baboons were from a pool of animals being used for bilharzia research at the two Institutes.

2.2 The approximate age of the animals was assessed from the status of the dentition and the eruption data of the teeth (Schultz, 1935 Ockerse, 1959; Virgadamo et al., 1972; Schwendeman et al., 1980). The vervet monkeys were given serial number 1 to 10, while the numbers of the baboons are those given in the bilharzia research study (Table 1). All the animals, including the ones from the bilharzia study were essentially healthy except two

vervet monkeys which were found to have lung congestion, but with no apparent brain damage.

B. Experimental Procedure for HRP retrograde axonal transport method for tracing neural connections from teeth.

2.3 The vervet monkeys were anaesthetized with Ketamine Hydrochloride (1ml/3kg body weight), while the baboons were anaesthetized with a mixture of 3ml of 2% Zylazine (Rompun) and 7.0ml of ketamine hydrochloride. The dose of the mixture was about 1ml/15kg body weight. Ketamine was found to be adequate for anaesthesia and the animals revived without undue problem.

2.4 Once the animal was anaesthetized, 20-30% HRP (Sigma Type VI) was injected in the periodontal ligament or tooth pulp of the test tooth or teeth types. The injections were carried out with 5-50µl Hamilton Microsyringe. The teeth types investigated were central and lateral incisors, canines and first and second molars, either maxillary or mandibular. Except in three monkeys and two baboons, injections of HRP were made in specific teeth types unilaterally, either in the right or left side of each dental quadrant. In the five animals three monkeys and two baboons two tooth/teeth types were injected in the right and left side of the opposite dental arch.

2.5 For the periodontal ligament, 50-100 $\mu$ l of HRP was injected and the needle was inserted in the periodontal ligament as apically as possible. The injection was given at a very slow rate. There was considerable backflow over the gingivae and the excess fluid was wiped with cotton wool. The injection into the periodontal ligament was made all around the tooth, buccally, medially, lingually, and distally requiring about six injections of 10 $\mu$ l each. In addition, in some animals, a mucosal flap was raised and a small "Window" was made in the buccal plate to the apex of the tooth. The buccal bone was drilled using a dental drill. This procedure was carried out in maxillary and mandibular central and lateral incisors, first and second molars and mandibular canine. Care was taken not to perforate the root. This procedure was referred to as apical injections. About 20 $\mu$ l of HRP was injected around the root apex and a small pledget of cotton wool or gelfoam soaked in HRP solution was placed in situ at the apex. The mucosal flap was replaced and stitched. In three animals one monkey and two baboons, a tooth or teeth were extracted, bleeding controlled and HRP solution was injected into the tooth socket. In the monkey, a cotton wool pledget in 30% HRP solution was left in situ in the socket. (Table 2a, 2b).

2.6 For the tooth pulp investigations, a cavity was made on the buccal surface of the selected tooth or teeth using a dental drill. Once the pulp was nearly



exposed (no frank bleeding) 5-20 $\mu$ l of HRP solution was gently injected into the pulp. Excess fluid was wiped and a few dry HRP grains were put on pulp. The cavity was sealed with dental cement (Table 2a, 2b).

2.7 A survival period of 48-72 hours was allowed for the vervet monkeys and 48-120 hours for the baboons. (In two baboons with survival of 120 hours, HRP was injected twice, day 1 and day 4. Table 2 shows the various teeth types injected with HRP in the periodontal ligament or dental pulp and respective survival periods allowed for retrograde HRP transport in monkeys (2a) and baboons 2(b).

2.8 Subsequently, the animals were deeply anaesthetised with sodium pentobarbital (60mg/kg body weight). In most of the animals, perfusion was transcardial, but in some of <sup>the</sup> monkeys and baboons from the bilharzia study, only the heads were perfused through the carotid artery. Heparin was injected intravenously prior to commencing perfusion. Using the gravimetric method, the animal was perfused rapidly with one litre of normal saline (pH 7.4) followed by a slow perfusion with one litre of fixative solution containing 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1M phosphate buffer at pH 7.4.

2.9 The animals were then decapitated and the calvaria opened-up in order to remove the brains and the trigeminal ganglia from the cranial cavity if fairly well



fixed or else left in situ if fixation was poor. The brains and right and left trigeminal ganglia<sup>(Fig.I,2)</sup> or the heads were left in buffered fixative (same as that used for perfusion with 30% buffered sucrose added) overnight at 4 degrees Centigrade. The brainstems were dissected from the brain between the level of superior colliculi and the middle of pons at the level of trigeminal nerve connection<sup>(Fig.I)</sup>. A nick was made ventrally on the right side of the brainstem segment in order to differentiate the two sides in the sections. The brainstem and trigeminal ganglia were left in buffered 10% sucrose for 1-4 days at 4 degrees Centigrade to allow for clearing of the fixative and provide cryoprotection.

2.10 The specimens were mounted on a freezing microtome stage with phosphate buffer at pH 7.4 and frozen sections cut serially at 50 $\mu$ m in the case of the trigeminal ganglion, and at 50-100 $\mu$ m in the case of the brainstem. The planes of sectioning were transverse and dorsoventral for the trigeminal ganglion, and coronal (rostro-caudal) for the brainstem.

2.11 Serial sections (10 at a time for the brainstem and 5 for the trigeminal ganglia) were collected in separate jars containing 0.1M phosphate buffer (pH 7.4) at 4-10 degrees Centigrade. Prior to the histochemical reaction, the sections were kept in the same buffer at 4.0 degrees Centigrade.

2.12 The histochemical reaction was carried out within 24-96 hours after sectioning, at which time the sections were rinsed in water several times to remove any traces of aldehydes and then processed for HRP precipitation reaction using the tetramethyl benzidine (TMB-Sigma) procedure as recommended by Mesulam (1978, 1982). The sections were then mounted on clean chrome-alum coated slides, air dried for 24-48 hours and counterstained with 1% neutral red (pH 3.3-4.8) for 2-3 minutes. Subsequently, the sections were dehydrated in graded concentrations of isopropyl alcohol or ethanol (from 70% to absolute) cleared in zylene and coverslips mounted with DPX. All the sections were examined within 24 hrs. The HRP reaction product was observed in a Leitz photomicroscope using bright field microscopy and photographs taken on black and white, and colour films.

2.13 Some frozen sections (about 10-15) were, however, not processed for HRP histochemical reaction with TMB for control. These sections were mounted on slides, air-dried for 24-48 hours and stained with neutral red. The sections were dehydrated, cleared and coverslips mounted in <sup>the</sup> same way as the TMB processed sections

### C. Electron Microscopy

2.14 Material for electron microscopy of the mesencephalic nucleus of trigeminal nerve was removed from the brainstem of a vervet monkey perfused with a

mixture of 10% glutaraldehyde and 4% paraformaldehyde. The areas around the periphery of the central gray matter were carefully dissected out in 1-2mm<sup>3</sup> blocks and postfixed with osmium tetroxide, dehydrated in graded alcohols and processed for araldite embedding. Thin (0.5  $\mu$ m) sections were stained with toluidine blue and examined with light microscope to ascertain the areas of cell clusters. Ultra thin sections were obtained and double stained with uranyl acetate for 30 min. and lead citrate for 5 min. The sections were observed in Carl-Zeiss (EM 952) electron microscope and electron micrographs were taken.

2.15 Some frozen sections (50-80 $\mu$ m) of trigeminal ganglia processed for HRP reaction product using the TMB procedure were postfixed in osmium tetroxide, dehydrated in graded alcohols and embedded in araldite. 0.5  $\mu$ m sections were cut and examined in<sup>a</sup> light microscope. The ultrathin sections were examined in the electron microscope (EM 952) and electron micrographs were taken.

#### D. Stereological Analysis of the mesencephalic nucleus and trigeminal ganglion.

2.16 The analysis of the mesencephalic nucleus of the trigeminal nerve and the trigeminal ganglion was carried out using the stereological techniques described by Weibel (1979, 1980); and Elias and Hyde, (1980). Serial transverse stratified sections of the brainstem and

trigeminal ganglion of the vervet monkey and olive baboon were analysed to obtain mean values of the volume density ( $V_V$ ), numerical density ( $N_V$ ) and total cell counts of the neurons of the mesencephalic nucleus and the trigeminal ganglion.

2.17 The technique used in this study to estimate the volume densities was that of point counting. Zeiss integrating graticule with, 100 points was used to carry out field by field analysis of section. The volume densities of the mesencephalic nucleus of the trigeminal nerve in the brainstem and the neurons of the trigeminal ganglion were estimated from the respective reference volume. The numerical density of the neurons was determined by counting neuron profiles on a defined area of the graticule at an appropriate magnification

2.18 The mathematical formulae computed by Weibel and Gomez (1962) and Weibel (1979, 1980) were applied to estimate the volume density and numerical density of the neurons from the raw data.

2.19 Although serial frozen sections of brainstem and ganglia obtained from the HRP studies may be adequate for the morphometric analysis, the thickness of the sections and short term stability of the neutral red stain made the observation of neurons on such preparations indistinct. To prepare paraffin-wax embedded sections, brainstem and trigeminal ganglion were obtained

from vervet monkeys and olive baboons perfused with 10% formalin. The animals were available in the Department of Human Anatomy and Institute of Primate Research.

2.20 Serial paraffin wax sections were cut from the brainstems of 7 monkeys and 4 baboons. The monkeys (2-4kg) were identified by letters A-G and 4 baboons, 3 adults (12 - 20kg) identified by numbers 1,2,3 and one young baboon (6kg) as No.4. The extent of the brainstem from the anterior aspect of the superior colliculi (the posterior commissure) to the middle of pons where the trigeminal nerve central branches connected was consistently ascertained. For ease of processing and dehydration, the brainstem was cut in three pieces comprising the segment of the superior colliculi, inferior colliculi and pons. The three segments were subsequently processed and sectioned separately (Fig.3a). The thickness of the sections obtained from the various brainstems ranged from 7 $\mu$ m to 20 $\mu$ m. All sections were collected from the brainstem where 20  $\mu$ m sections were transected. Every 5th, or 10th section was collected when the sections were 7 $\mu$ m and 10 $\mu$ m. The numbering of the sections was consecutive from the rostral limit of the superior colliculi to the caudal limit of pons. The plane of sectioning was transverse (coronal) through the midbrain and pons. All the sections were stained with Haematoxylin and Eosin stains. In two monkeys (A and C) and two baboons ( 1 and 4 ), the volume of the whole

brainstem was measured prior to transection using the water displacement method (Scherle, 1970).

2.21 Ten to 20 equidistantly spaced serial sections of the brainstem were analysed at the magnification of  $\times 35$ . In a section analysed, the number of points falling on the mesencephalic neurons as compared to the total number of points on the section gives the volume density of mesencephalic neurons ( $V_{V_{mes}}$ ). The number of profiles of mesencephalic neurons in the section were counted. The area of graticule within the 100 points was calibrated using a slide etched scale at the magnification  $\times 35$ . The total area of the section was calculated by multiplying the number of test fields of a section by the test area ( $\text{mm}^2$ ) of each field at the magnification used (Fig. 3b). The area of the section was counter-checked by superimposing the outline of the large brainstem sections over a graph paper. The number of small squares ( $\text{one mm}^2$ ) falling within the outline of the section were counted (Fig. 3b). The number of cells per unit area ( $N_A$ ) was obtained by dividing the number of cells in the section by the total area.

2.22 Paraffin-wax sections were cut from four trigeminal ganglia (right and/or left) of 3 monkeys and 2 baboons. The ganglia, identified as (a) and (b) were right or left from monkey A and B ganglia (c) and (d) were right and left from monkey C. Right and left

ganglia of one adult baboon (No.1, 16kg) and one juvenile baboon (No. 4, 6kg) were transected. The ganglia were trimmed so that the three divisions of the trigeminal nerve could be easily seen as short stumps emerging from the grayish crescent shaped band (Fig. 4a). The central fibres were also cut along the edge of the ganglion. The motor root was visible on the ventral aspect of the ganglion. All sections of a ganglion were collected. The thickness of the sections ranged from 7 to 10um, and they were cut in horizontal plane, from dorsal to ventral aspect.

2.23 The sections were stained with Haematoxylin and Eosin. The reference area for analysis of the "ganglionic" region was up to the periphery of the band of neurons (Fig. 4a). The volumes of all the ganglia were measured prior to sectioning using the water displacement method (Scherle, 1970).

2.24 Five to 10 equidistantly spaced serial paraffin-wax sections of the ganglia were analysed at the magnification of X100. The number of points falling on the neurons and the rest of the structures such as axons, dendrites and blood vessels was counted (Fig. 4b). The volume density of the neurons in each test field was calculated and the mean volume density of neurons in each section was obtained. From all the sections analysed for one ganglion, the mean volume density of neurons in the



ganglion was calculated, noting the variation from section to section. The number of neuron profiles within the area of the grid were counted for each field and subsequently the total number of neurons in a section were calculated (Fig. 4b). The area of the section was obtained by the number of fields multiplied by the test area. The number per unit area = total number of neurons in the section/total test area of the section.

2.25 The diameters of cell profiles of 100 mesencephalic neurons and 100 trigeminal ganglion neurons of 7 monkeys and 4 baboons respectively were measured. The neurons with a clearly visible nucleus were measured across the cell cytoplasm using a calibrated grid in one eye - piece at the magnification of X400. The measurements of diameters were made in neurons at various levels along the rostro-caudal extent of mesencephalic nucleus and at various levels in the trigeminal ganglion. Mean value of the diameter and S.D. for the mesencephalic and ganglion neurons was calculated for the monkey and the baboon.

2.26 The formula derived by Weibel and Gomez (1962) used for estimation of the number of cells per unit volume ( $N_V$ ) is

$$N_V = \frac{N_A^{3/2}}{\beta \cdot V_V^{1/2}} \text{-----a}$$



Where  $\beta = 1$  for spherical particles such as neurons.  $V_V$  is the volume proportion of the neurons in the brainstem and ganglion respectively,  $N_A$  is number per unit area.  $N_V$  was also estimated by using the formula of Weibel (1979, 1980).

$$N_V = \frac{N_A}{\bar{D} + t} \text{-----} b$$

Where  $\bar{D}$  is the mean caliper diameter of the neurons and  $t$  is section thickness. The estimation of  $\bar{D}$  requires population size distribution plots which were not done. The  $\bar{D}$  was assumed to be reasonably close to  $D$ , the mean diameter. This may not be too inaccurate as the neurons approximate to spheres very closely as they have a shape coefficient  $\beta$  of 1 (Weibel, 1979, 1980).

2.27 The mean values for the volume density  $V_V$  number per unit area  $N_V$  and numerical density  $N_V - a$  and  $N_V - b$  of the mesencephalic neurons and trigeminal ganglion neurons in monkeys and baboons were calculated from values from analysis of individual sections of each of the brainstem and ganglion.

2.28 The total number of mesencephalic neurons and trigeminal ganglion neurons in monkeys and baboons were subsequently estimated ( $N_{T(est)}$ ) from the numerical density ( $N_V - a$ ) and the volume of the brainstem and ganglion respectively using the formula  $N_{T(est)} = V_V \times \text{Volume}$ . Correction to the measured volume of the

ganglion was made for the non-ganglionic tissue such as the three divisions of the trigeminal nerve by determining the volume proportion of the non-ganglionic tissue as compared to the whole ganglion (Fig 4a).

2.29 In the paraffin-wax embedded serial sections of the brainstem of the 7 monkeys and 4 baboons, total cell counts ( T count ) of mesencephalic neurons were also obtained by counting the number of neurons with a clearly visible nucleoli in every collected section, bilaterally, from rostral to caudal extent of the mesencephalic nucleus.

2.30 In the paraffin wax sections of the ganglia where every section was collected the total number of neurons with clearly visible nucleus in a section was counted in five equidistantly spaced sections and a mean value obtained for one section. The total number of neurons (T count) in the ganglion was obtained by multiplying the total number of sections of a ganglion with the mean number of neurons per section.

2.31 The serial frozen sections of the trigeminal ganglia of six monkeys from the HRP studies were analysed to obtain the volume densities of HRP labelled and unlabelled neurons. The volume percentage of the labelled neurons out of the total volume of the neurons was calculated.

2.32 Shrinkage constant for the monkey brainstem and trigeminal ganglion was estimated. Rectangular blocks of fresh tissues were measured on two sides. They were left in 10% formalin for two days and measured again. The tissues were then dehydrated in <sup>graded</sup> alcohols and embedded in molten wax and measured at each stage to note the change from the fresh to the final stages of embedding.

Table 1 showing wt., sex, approximate age and dentition of the monkeys and baboons used in the study

Monkeys (NOS)	Wt (Kg)	Sex	Age	Dentition
1	3.0	Adult female	6 yrs +	Perm. Dentition
2	1.5	Young male	2-3 yrs	Mixed Dentition
3	2.0	Young female	3-4 yrs	M <sup>3</sup> unerupted
4	2.9	Adult male	6 yrs	Perm. Dentition
5	2.2	Adult male	6 yrs	"
6	2.7	Adult male	6 + yrs	"
7	3.1	Adult female	6 + yrs	"
8	2.7	Adult male	6 yrs	"
9	2.1	Young female	4 yrs	M <sup>3</sup> unerupted
10	1.8	Young female	4-5 yrs	"

Baboons (Nos)	Wt	Sex	Age	Dentition
879	15	Adult female	8 yrs +	Perm. Dentition
880	17	Adult male	8 yrs +	"
881	13	Young male	6-7 yrs	M <sup>3</sup> unerupted
863	12	Young female	5-6 yrs	M <sup>3</sup> unerupted
864	10	Young female	5-6 yrs	"
865	13	Young female	5-6 yrs	"
866	12	Young male	4-5 yrs	"
30	26	Adult male	8 yrs ++	Perm. Dentition
33	14.5	Adult female	8 yrs +	"
58	6.3	Juvenile male	2 yrs +	M <sub>1</sub> erupting
59	5.5	Juvenile male	- 2 yrs	Dec. Dentition
60	4.25	Juvenile female	- 2 years	"
62	3.9	Juvenile female	- 2 years	"

Table 2 showing the various teeth types injected with HRP in the periodontal ligament or dental pulp and respective survival periods allowed for retrograde HRP transport in monkeys (2a) and baboons 2(b)

Table 2a - Vervet monkeys

NO	Teeth - Periodontal ligament/gingivae (pdl) or pulp	Survival Period
1	Rt Mand $M_1$ and $M_2$ (pdl) socket following Extr.	53 hrs
* 2	Rt Max $I_1+I_2$ (pdl) intact LT Mand $M_1$ (pulp) - buccal cavity	48 hrs
3	LT Max canine (pdl) intact Rt Mand $M_1+M_2$ (pdl) intact	48 hrs
* 4	Rt Mand $I_1I_2$ (pdl) intact + apical "window" LT Max $M_1+M_2$ (pdl) intact + apical "window"	48 hrs
5	Rt Mand canine (pdl) intact + apical window	52 hrs
6	Rt Mand $I_1I_2$ (pdl) intact + apical "window" 2 injections of HRP, day 1 and day 2	72 hrs
* 7	Rt Mand $M_1+M_2$ (pdl) intact + apical window - 2 injections of HRP LT Max canine (pulp) buccal cavity	72 hrs
8	Lt Max canine (pdl) intact	48 hrs
9	Rt Max $M_1+M_2$ (pdl) intact	48 hrs
10	Rt Mand $I_1+I_2$ (pdl) intact	48 hrs

\* Monkey in which two tooth/teeth types were injected in the right and left sides of the opposing dental arch.

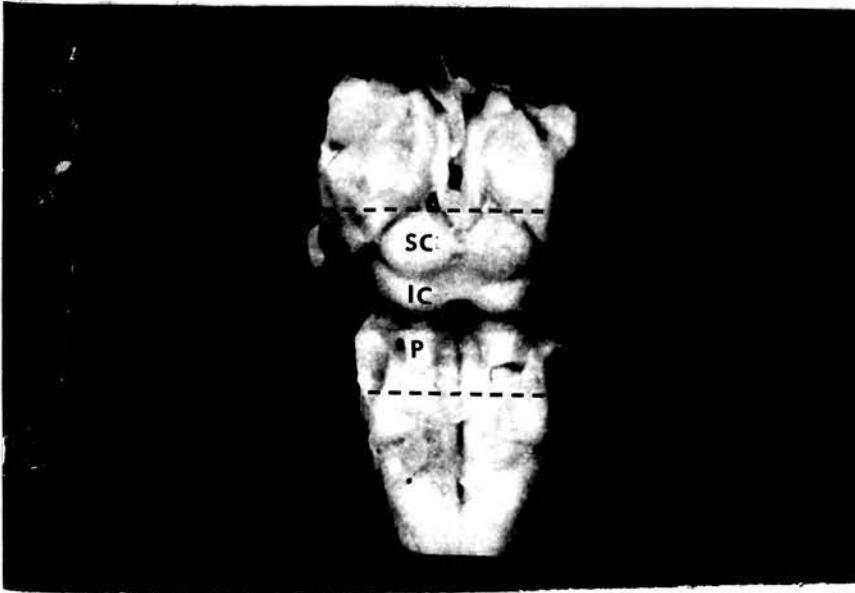
Table 2b Olive baboons

NO	Teeth - periodontal ligament/gingival (pdl) or pulp	Survival Period
879	Rt Max canine (pdl) intact	72 hrs
880	Rt Max I <sup>1</sup> +I <sup>2</sup> (pdl) socket following extraction	72 hrs
881	LT Mand M <sub>1</sub> M <sub>2</sub> (pdl) Socket " "	72 hrs
863	LT Max I <sup>1</sup> I <sup>2</sup> (pdl) intact	120 hrs
*864	Rt Mand M <sub>1</sub> +M <sub>2</sub> (pdl) intact	72 hrs
	LT Max canine (pdl) intact	
865	Rt Mand I <sub>1</sub> +I <sub>2</sub> (pdl) intact	72 hrs
*866	LT Max M <sup>1</sup> +M <sup>2</sup> (pdl) intact	120 hrs
	Rt Mand canine (pdl) intact	
30	LT Max I <sup>1</sup> +I <sup>2</sup> (pulp) buccal cavity	48 hrs
33	LT Mand M <sub>1</sub> (pulp) buccal cavity	48 hrs
58	LT Mand M <sub>1</sub> (pdl) intact	48 hrs
59	LT Mand Deciduous canine (pdl) intact	48 hrs
60	LT Mand Deciduous I <sub>1</sub> +I <sub>2</sub> (pulp)	48 hrs
62	LT Max Deciduous I <sup>1</sup> +I <sup>2</sup> (pdl) intact	48 hrs

\*Baboon in which two tooth/teeth types were injected in the right and left sides of the opposing dental arch.



(i)



(ii)

Figure 1. Photographs showing (i) the ventral aspect of the brain of the vervet monkey. The broken lines indicate the approximate region of the brainstem sectioned X1.5 (ii) the dorsal aspect of the brainstem of the vervet monkey. The lines indicate the levels at which the brainstem was cut. X 1.5. Superior colliculi (SC) Inferior colliculi (IC), Pons (P).

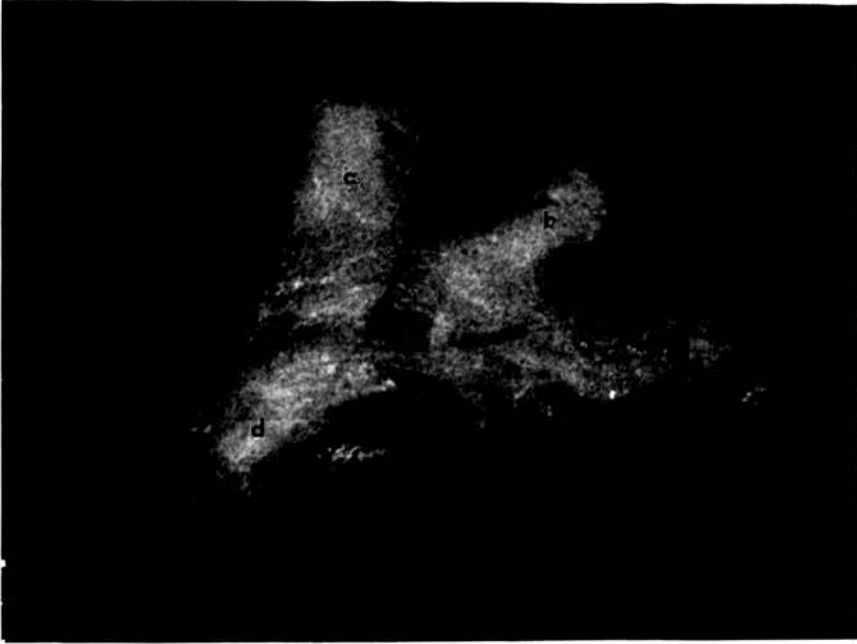


Figure 2. Photograph showing the ventral aspect of the trigeminal ganglion of vervet monkey. X 2.

(a) Ophthalmic branch (b) maxillary branch  
(c) mandibular branch (d) central root.



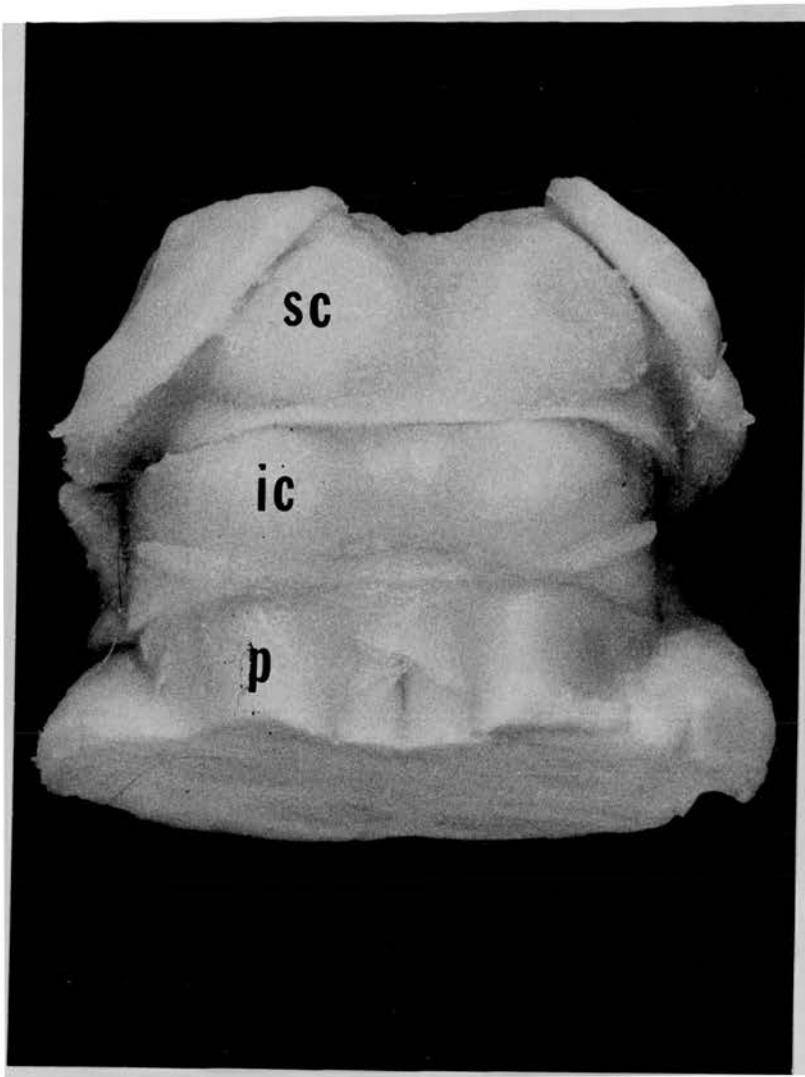


Figure 3a. Photograph showing the dorsal view of the brainstem of baboon in three segments comprising the superior colliculi (SC), inferior colliculi (IC) and pons (P). X 8.

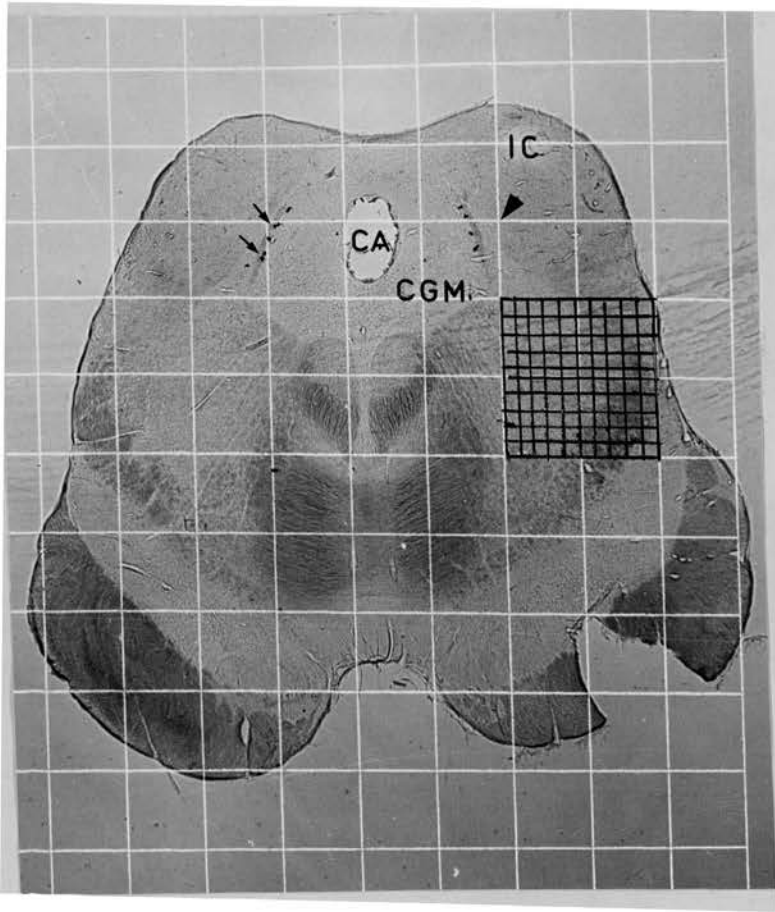
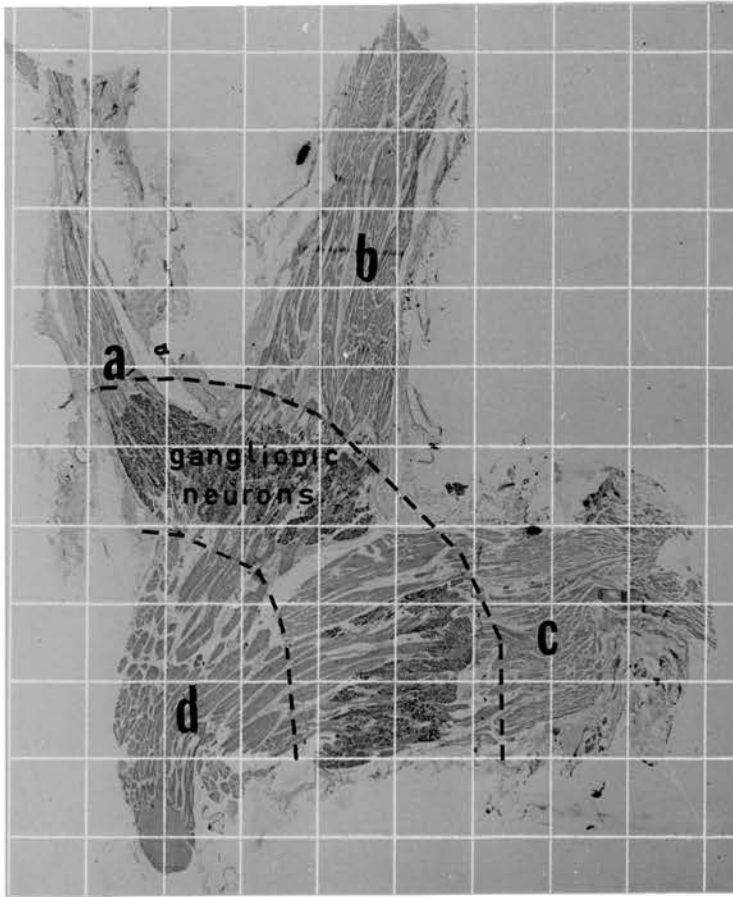


Figure 3b. Photomacrograph of transverse sectional profile through the midbrain of baboon with an overlay grid to illustrate the method of point counting. (4) Arrowhead shows the position of a point. The shaded area shows an "area of field" as for field by field analysis of section. Arrows show the approximate position of mesencephalic neurons. Inferior colliculi (IC), Cerebral aqueduct (CA), central grey matter (CGM). X6.



**Figure 4a.** Photograph of a transverse sectional profile of the trigeminal ganglion of the vervet monkey with an overlay grid to show how the analysis of volume proportion of "ganglionic" and "non-ganglionic" tissue was carried on. The approximate region of the "ganglionic" tissue is outlined. X10.  
 a - ophthalmic branch, b - maxillary branch  
 c - mandibular branch, d - central fibres.

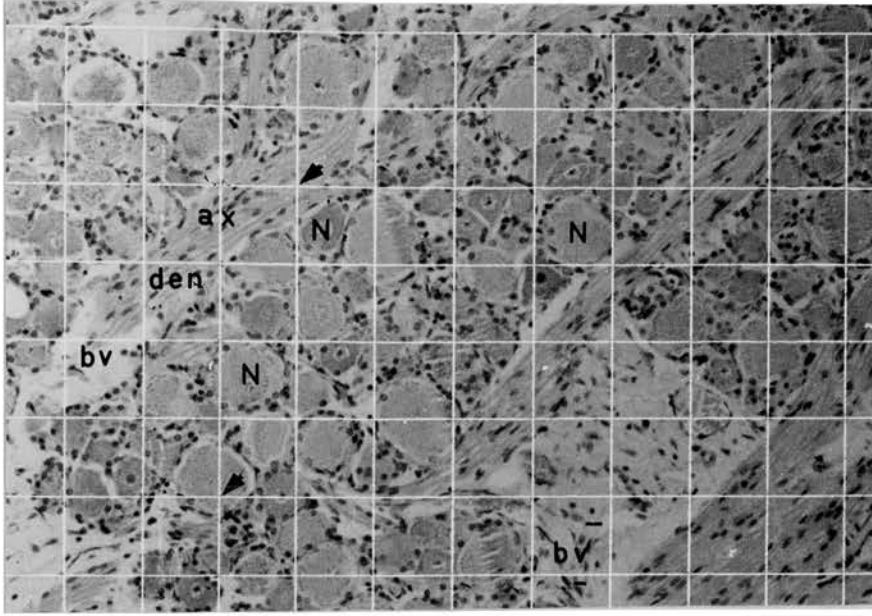


Figure 4b. Photomicrograph of a paraffin wax section of trigeminal ganglion of baboon with an overlay grid to illustrate the method of point counting used in the analysis of the volume proportions of the neurons and non neuronal tissue and also profiles on area counts for obtaining numerical density of the neurons. X125. Arrowhead shows the position of a point, Neurons (N), blood vessel (bv), (ax) axon, (den) dendrites.

### 3. OBSERVATIONS AND RESULTS

#### 3 A. Structural Organization of the Mesencephalic Nucleus of the Trigeminal Nerve in the Vervet Monkey and Olive Baboon

3.1. The mesencephalic nucleus of the trigeminal nerve in the vervet monkey and olive baboon was found to extend as a band of neurons along the rostro-caudal extent of the midbrain from the level of the superior colliculi rostrally to the anterior part of pons caudally (Fig. 5). The midbrain is traversed in its central region by the cerebral aqueduct which is surrounded by the periaqueductal central gray matter (Figs,6,7). The colliculi form the tectum dorsally, overlying the tegmentum, while the substantia nigra and the cerebral peduncles form the ventral part of the midbrain. (Figs. 5,6,7).

3.2. The pons begins at the caudal limit of the inferior colliculi (Fig.5). The ventral part of pons is composed mainly of the middle cerebellar peduncles (Figs.8,9). The floor of the fourth ventricle lies dorsally over the pontine reticular gray matter, bounded laterally by the superior cerebellar peduncles and above by the superior medullary velum. (Figs. 8,9).

3.3 At the level of the superior colliculi, rostrally, medium and large spherical mesencephalic neurons were

found lying dorsally along the margin of the periaqueductal central gray matter (Fig.10). Progressing caudally, the neurons were found to occur either singly or in small clusters dispersed along the dorsal and dorso-lateral margin of the central gray matter (Figs 6, 7).

3.4 The tract of the mesencephalic nucleus forms an attenuated bundle that lies adjacent to the mesencephalic neurons along the lateral margin of the central gray matter when observed in transverse sectional profiles (Figs.6,7,8). The mesencephalic neurons are generally found medial to the tract although some neurons are also seen within the tract itself. The nucleus of the oculomotor nerve lies in the gray matter ventral to the aqueduct at the level of the superior colliculi (Figs. 6, 7).

3.5 At the level of the inferior colliculi, the mesencephalic neurons are found to lie dorso-laterally and at the caudal limit of the inferior colliculi, the neurons were located on the lateral aspect of the margin of the central gray matter (Figs. 6,7,8). The mesencephalic neurons lie along and often within the tract of the mesencephalic nucleus at the caudal limit of the inferior colliculi. The mesencephalic tract forms a distinct crescent shaped band on the lateral aspect of central gray matter (Figs.6,7,8). The fibres of the

tract run ventro-laterally towards the pons. The nucleus of the trochlear nerve is found in the gray matter ventral to the aqueduct at the level of the inferior colliculi (Figs.6, 7).

3.6. At the junction of the inferior colliculi and pons, the trigeminal mesencephalic neurons were seen to aggregate on the dorso-lateral aspect of the gray matter around the floor of the fourth ventricle and medial to the caudal part of inferior colliculus (Fig.8). In this region the mesencephalic tract is observed as a prominent bundle located dorsal and medial to the superior cerebellar peduncle (Figs.8a,b).

3.7. In pons, the mesencephalic neurons lie principally on the dorso-lateral aspect of the floor of the fourth ventricle close to the superior cerebellar peduncles and medial to the tract of the mesencephalic nucleus (Figs 9a,b). The neurons were found on the dorso-lateral aspect of the locus coeruleus up to the level of the motor nucleus of the trigeminal nerve. The main sensory nucleus of trigeminal nerve is located laterally in pons (Figs.9ab).

3.8. Apart from the difference in size, no major differences in the topographic and cytoarchitectural organization of the brainstem of the vervet monkey and olive baboon were observed.

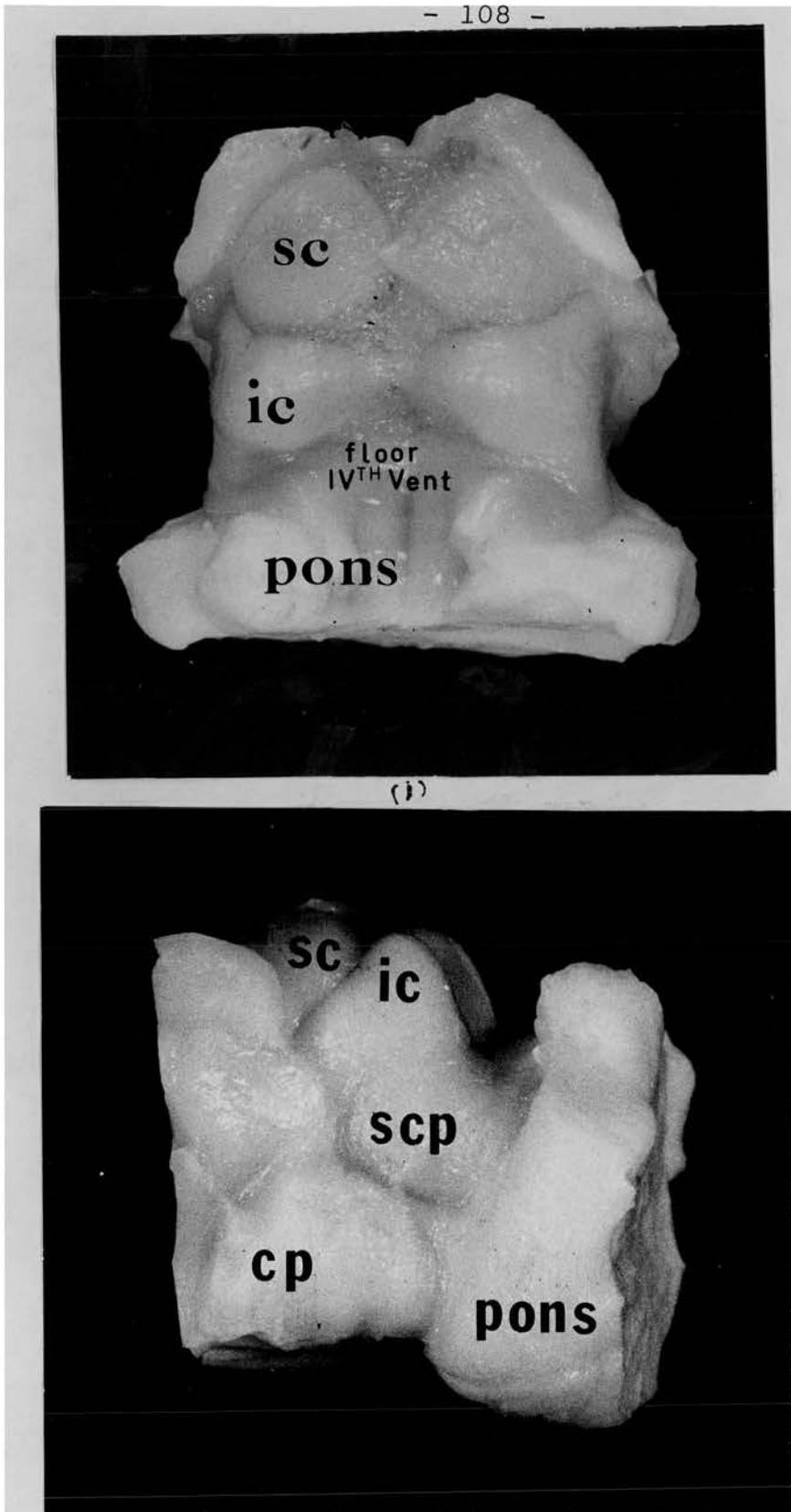
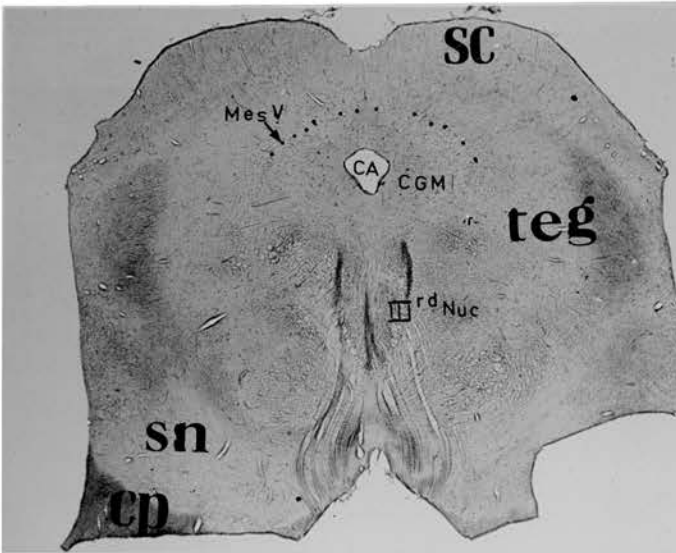


Figure 5. Photographs showing the (i) dorsal and (ii) lateral aspects of the brainstem of the vervet monkey x 8.5.

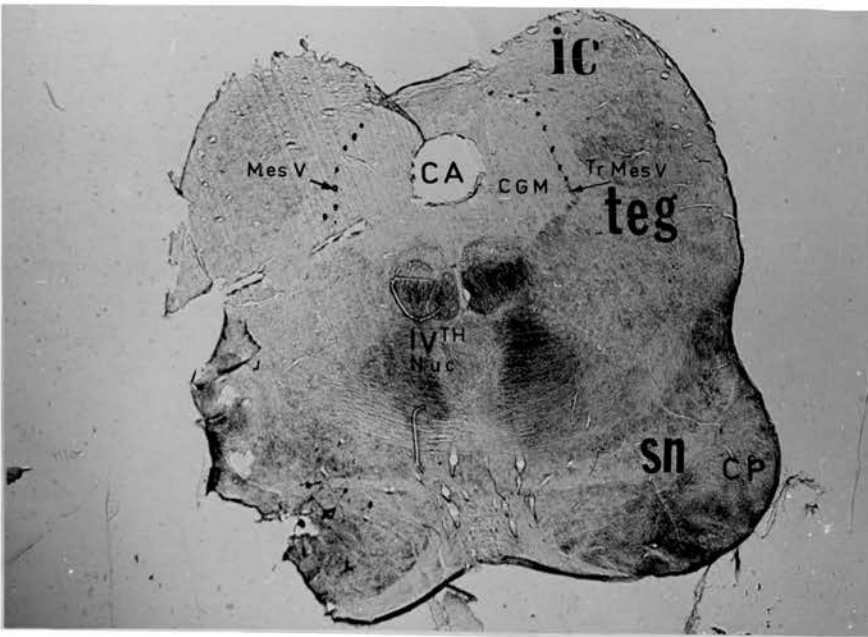
Superior colliculi (SC) Inferior colliculi (ic)  
Superior cerebellar peduncle (SCP), Fourth  
ventricle (IV<sup>th</sup> vent.) cerebral peduncle (CP)





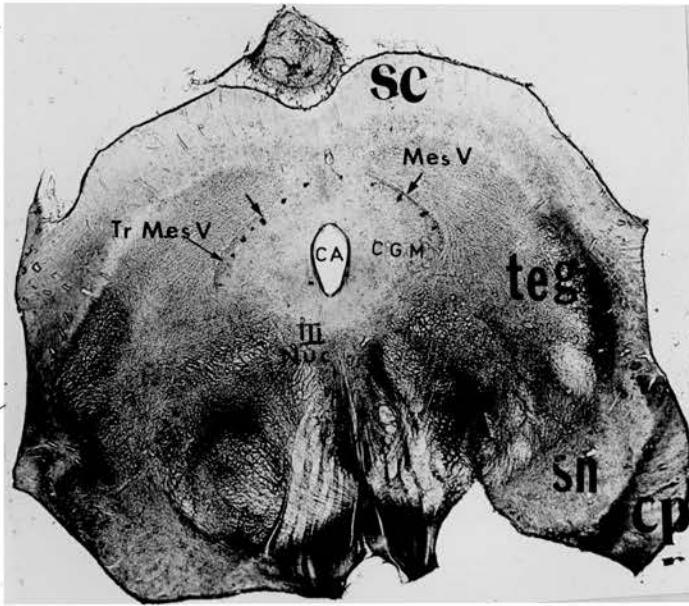
teg- Tegmentum  
CA - Cerebral aqueduct  
CGM - Central gray matter  
SN - Substantia nigra  
CP - Cerebral peduncle  
Tr - Mes V - Tract of  
mesencephalic nucleus  
III<sup>rd</sup> Nuc. - Oculomotor  
nucleus.  
IV<sup>th</sup> Nuc. - Trochlear  
nucleus

(i)

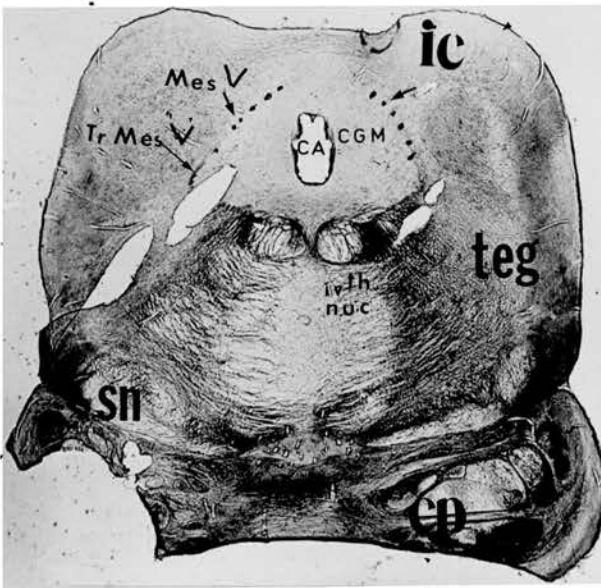


(ii)

Figure 6. Photomicrographs of the transverse sectional profiles of frozen sections (100µm) of the midbrain of vervet monkey at the level of (i) superior colliculi (SC) and (ii) inferior colliculi (IC) to show the position of the mesencephalic neurons (Mes V). X 7

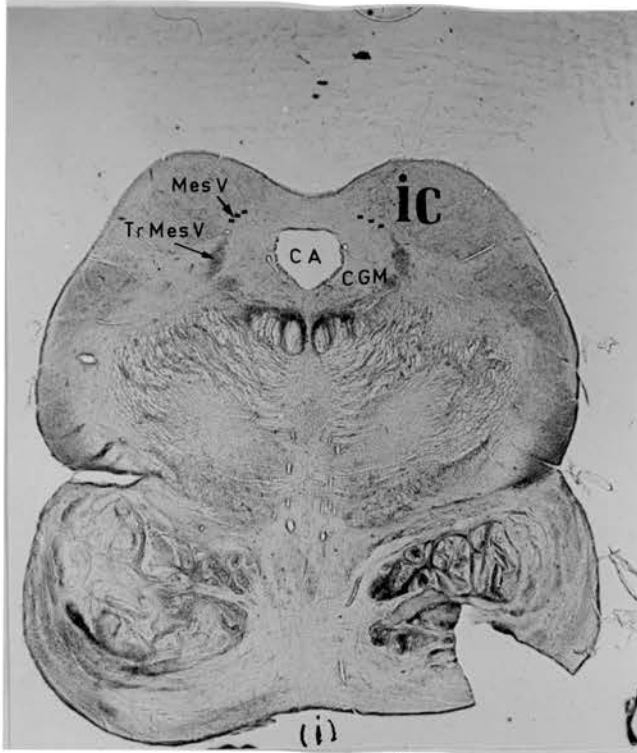


(i)



(ii)

Figure 7. Photomicrographs of the transverse sectional profiles of frozen sections (100 $\mu$ m) of the midbrain of the baboon at the level of the (i) superior colliculi (SC) and (ii) inferior colliculi (IC) to show the position of the mesencephalic neurons (Mes V). x.5.



CA - Cerebral aqueduct  
CGM - Cebtral gray matter  
rgm - reticular pontine  
gray matter

Tr-MesV- Tract of  
Mesencephalic  
nucleus

MCP - Middle cerebral  
Peduncle

IV<sup>th</sup> Vent - Fourth Ventricle

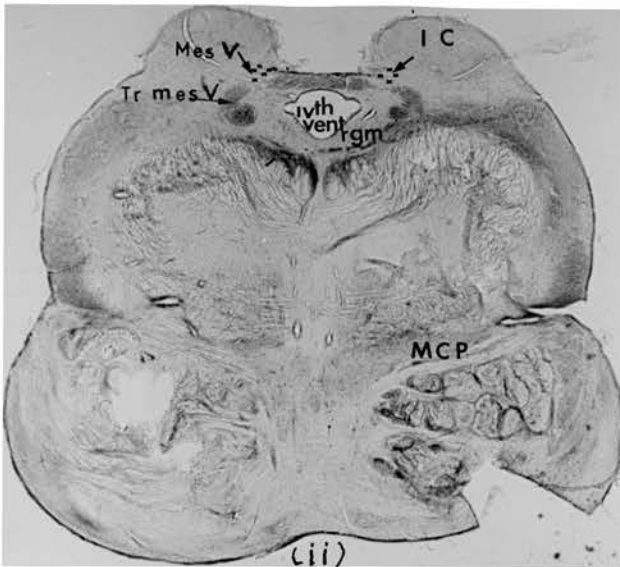


Figure 8. Photomacrographs of the transverse sectional profiles of frozen sections (100µm) of the brainstem of baboon (i) at the caudal aspect of inferior colliculi (IC) and (ii) rostral aspect of pons to show the position of the mesencephalic neurons (Mes V) x5.



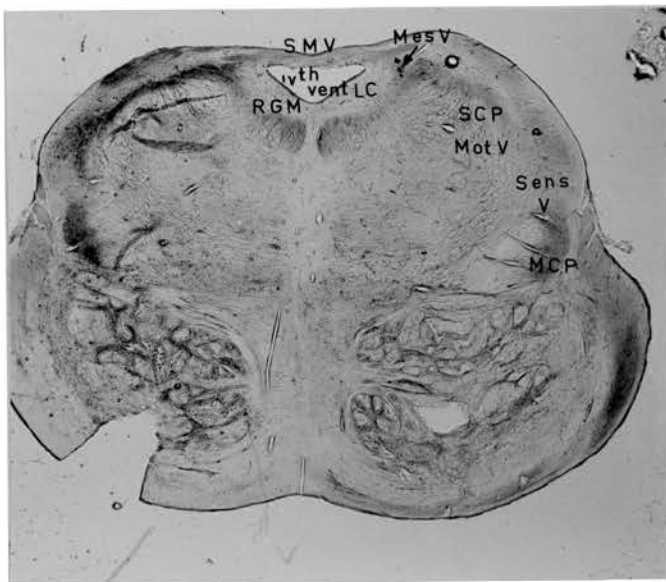
(i)

SMV - Superior Medullary Vellum

IV<sup>th</sup> Vent- Fourth Ventricle  
SCP- Superior Cerebral Peduncle

MCP - Middle Cerebral Peduncle

RGM Reticular pontine gray matter



(ii)

Mot V - Motor nucleus of Trigeminal Nerve

Sens V - Sensory nucleus of Trigeminal Nerve

L C - Locus coeruleus

Tr. Mes V - Tract of mesencephalic nucleus

Figure 9. Photomicrographs of transverse sectional profiles of frozen sections (100um) of the pons (i) of vervet monkey (x7) and (ii) baboon (x4) to show the position of the mesencephalic neurons (mes V).

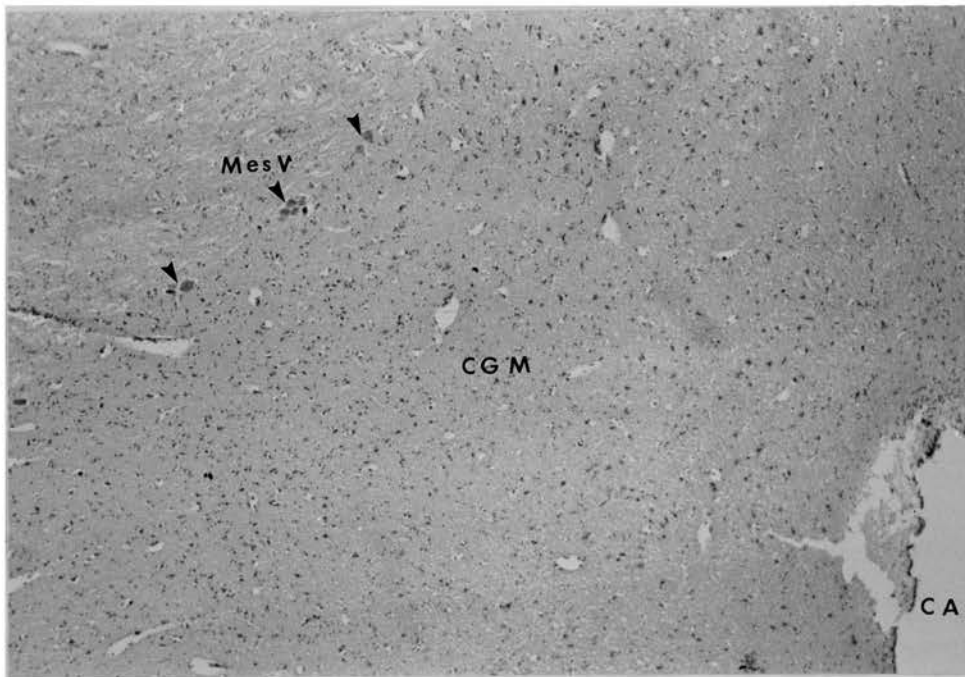


Figure 10. Photomicrograph of paraffin wax section (10 $\mu$ m) of midbrain (T.S.) of baboon showing the mesencephalic neurons (Mes V) in the periphery of the central gray matter (CGM) which surrounds the cerebral aqueduct (CA). H/E stain x50.

3.9. The mesencephalic neurons of the trigeminal nerve were densely staining mainly large and medium sized unipolar cells (Figs. 11ab). The mesencephalic neurons were more chromophilic than the surrounding cells and were located in the periphery of the central gray matter medial to the mesencephalic tract of trigeminal nerve. The mesencephalic neurons were generally round to oval in shape, medium to large with the diameter of the individual cell bodies ranging from about 30-60 $\mu$ m (Figs. 11ab). The neuron cell body has an incomplete ring of satellite cells made up of the surrounding glial cells, such that the cell body comes into direct contact with the neuropil element (Figs. 11a,b). The cell bodies of mesencephalic neurons contain Nissl granules and a large single nucleus with a prominent nucleolus. The Nissl substance forms fine evenly distributed granules with slight condensation at the periphery. The nucleus is large with a well defined nuclear margin, generally lying in the centre of cells but often having an eccentric position (Figs. 11a,b).

3.10. The unipolar process of the cell appears to divide into a central and peripheral process, both of which turn caudally forming the tract of the mesencephalic nucleus. The tract of the mesencephalic nucleus appears as myelinated fibres transversely cut lying lateral to the neuron cell bodies (Figs. 11a,b). The peripheral and central processes extend caudally

along the tract although the two processes are not clearly defined in the tract.

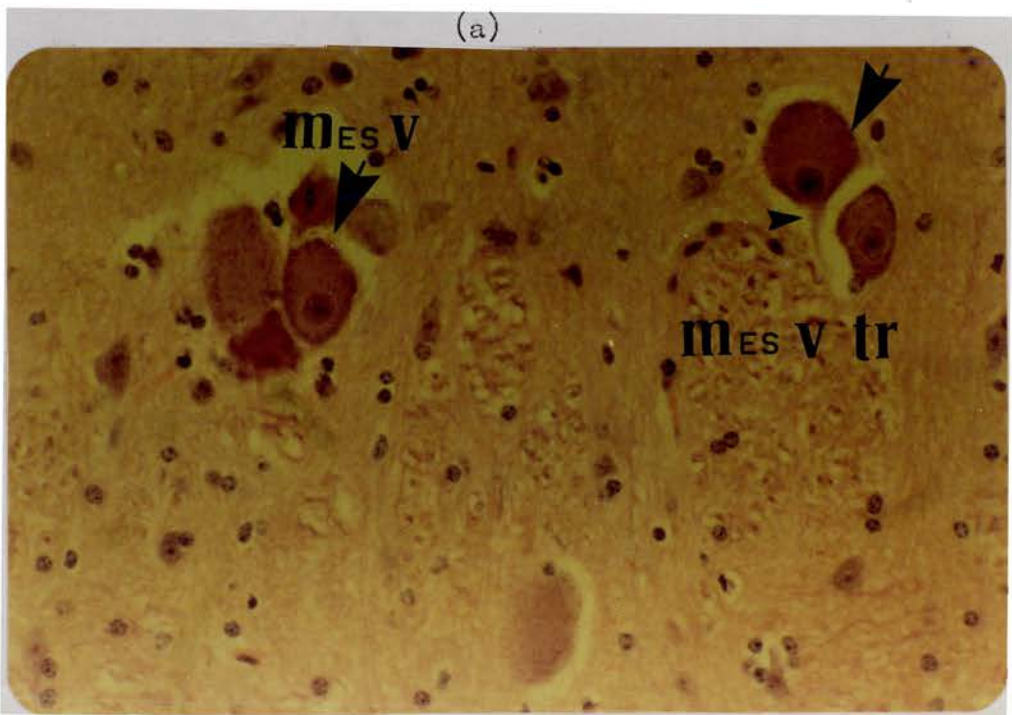
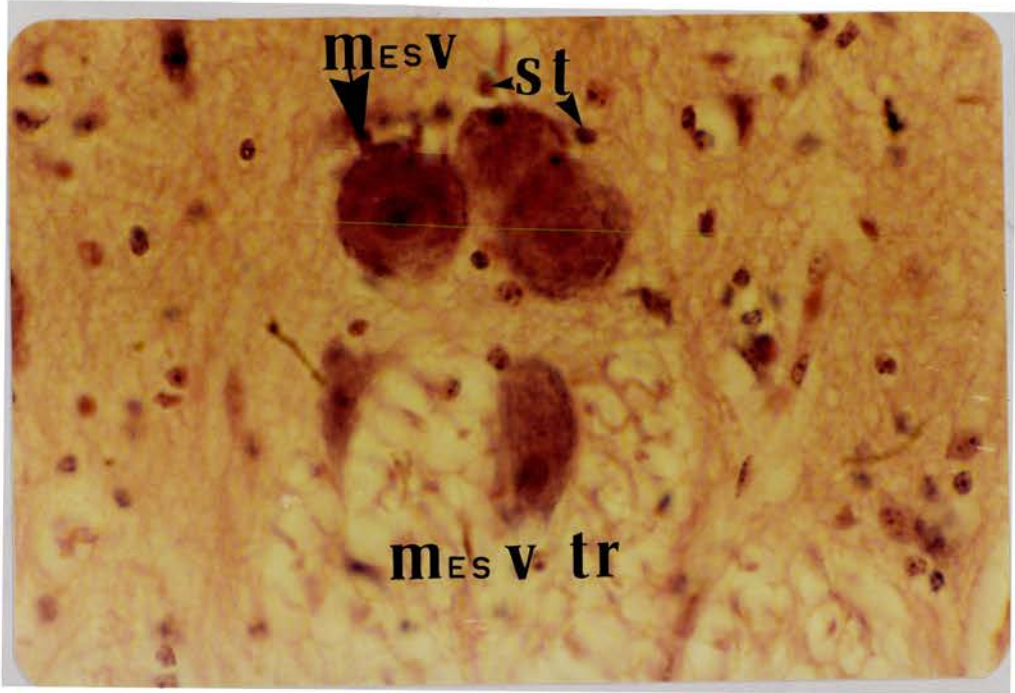
3.11 In the midbrain, the mesencephalic neurons were either single or in clusters of 2-9 cells dispersed along the periphery of the central gray matter. (Fig. 10, 11, 12). Some of the cells in the cluster appeared to show soma-soma contacts where the two mesencephalic neurons were observed to have contacts between the adjacent cell membranes (Fig. 13). An area of soma-soma contact between the mesencephalic neurons is shown in electronmicrographs where the cell membranes are seen to be in contact (Figs 14, 15). The area of the plasmolemmas of the apposed cells is characterised by macula adherens **gap** junction.

3.12. In the caudal part of the inferior colliculi, the mesencephalic neurons became sparse and the neuron cell bodies were found within the tract of the mesencephalic nucleus. These "intrafascicular neurons" were fusiform in shape and were about 60-70  $\mu\text{m}$  along the major axis of the cell (Fig. 16ab). The mesencephalic neurons had a nucleus which was either centrally placed or eccentric in position and the neurons were bipolar or multipolar (Fig. 16b).

3.13. In the rostral part of the pons and at the junction of the inferior colliculi, the mesencephalic neurons were

organized either in large clusters of 5-9 cells or as aggregation of large numbers of neurons. The cells were medium to large, spherical or oval and were located on the dorso-lateral aspect of the floor of the fourth ventricle (Fig. 17a,b).





(b)

Figure 11. Photomicrographs of paraffin wax sections (10-20 $\mu$ m) of midbrain (T.S.) of (a) vervet monkey and (b) olive baboon showing the mesencephalic neurons (Mes V) lying adjacent to the tract of mesencephalic nucleus (Mes V-tr). The Mes V neurons have Nissl granules, a nucleus with prominent nucleolus. A unipolar cell process (arrowhead) is seen in (b). H/E stain (a) x600 (b) x 300. Satellite cell (St)

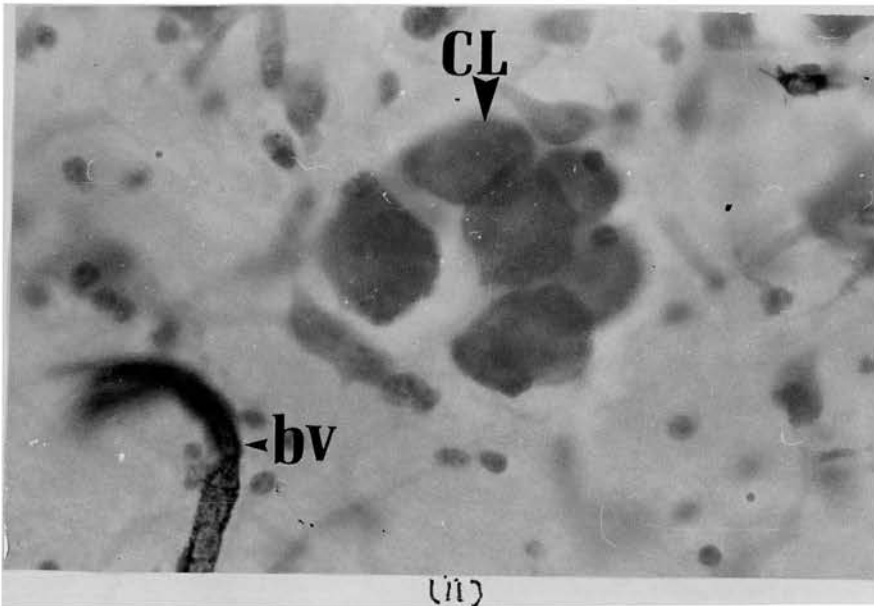
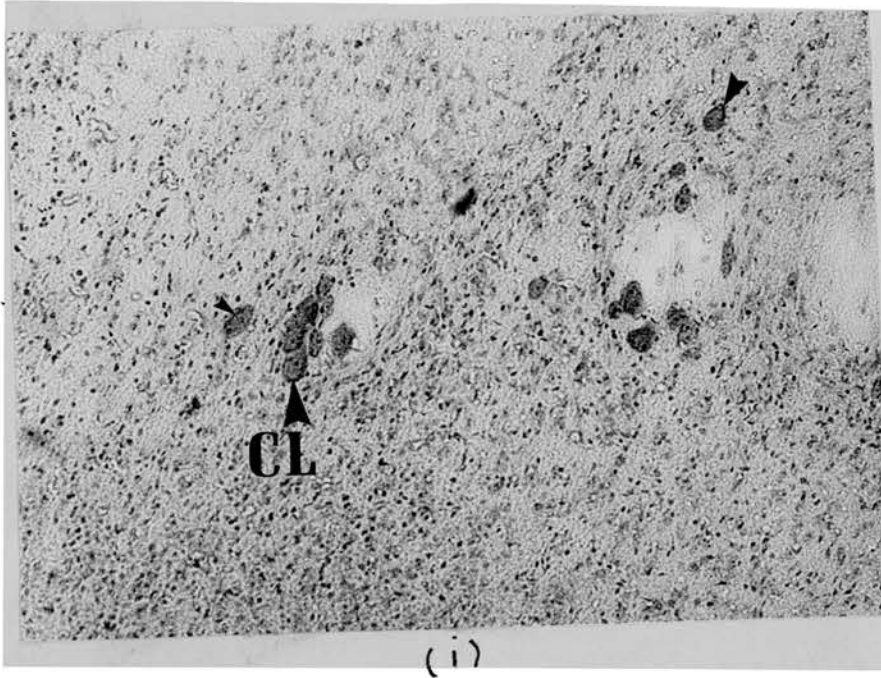
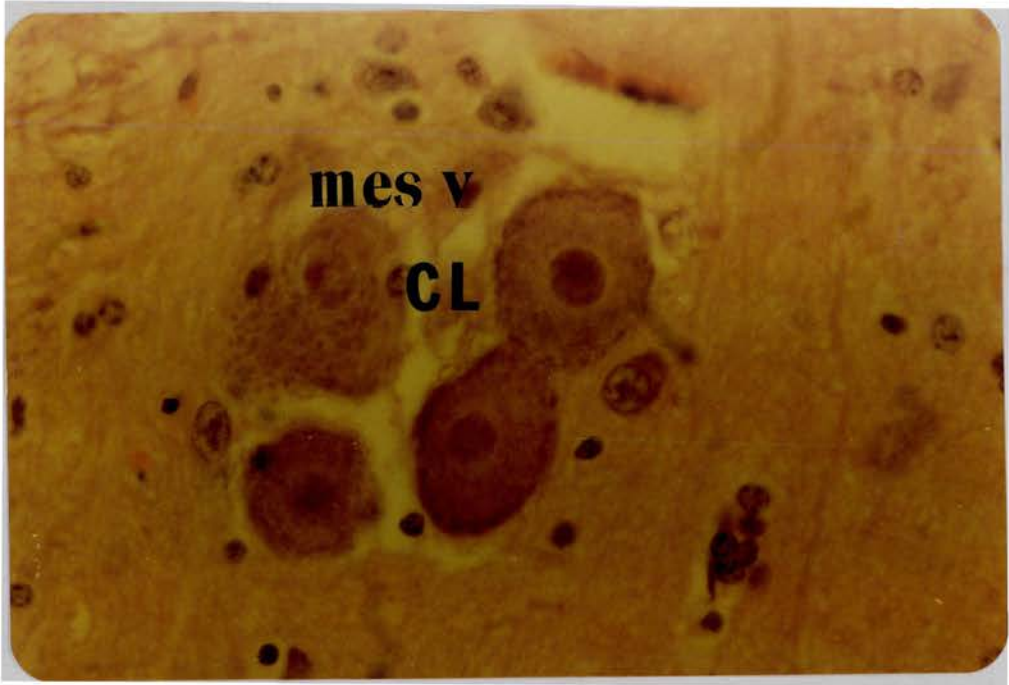
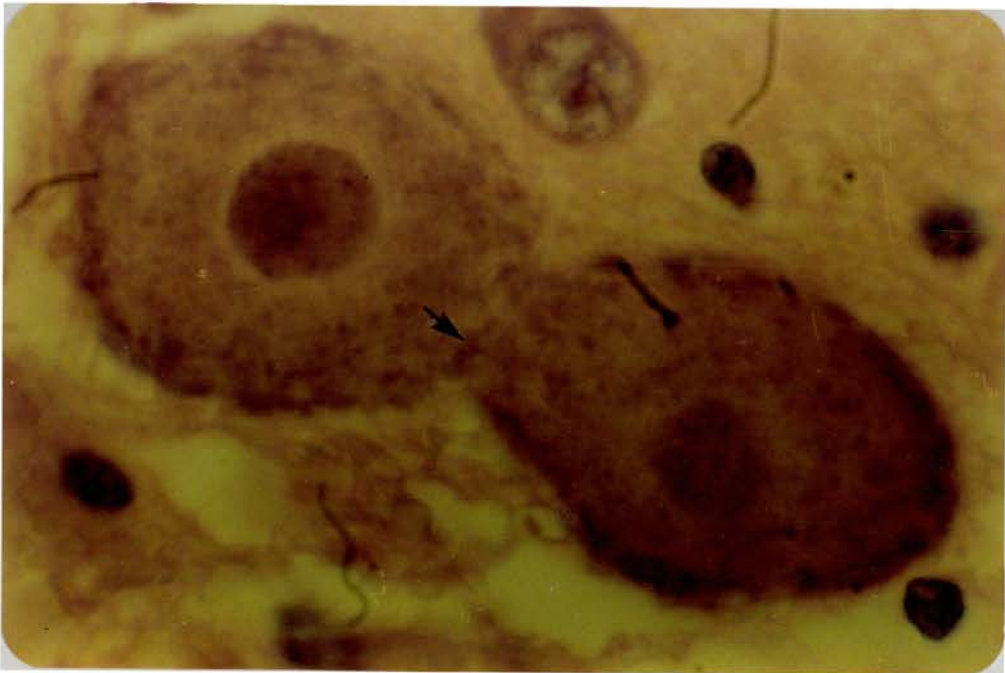


Figure 12. Photomicrographs of (i) paraffin wax section (10 $\mu$ m) and (ii) frozen section (100 $\mu$ m) of midbrain of monkey (T.S.) showing single neurons (arrowhead) and clusters of mesencephalic neurons (CL), blood vessel (bv). (i) H/E stain x 125 (ii) Neutral red stain x 300.



(i)



(ii)

Figure 13. Photomicrographs of paraffin wax section (10 $\mu$ m) of baboon mesencephalic neurons (Mes V) showing (i) a cluster of neurons (CL) (ii) with two of the neurons in apparent soma-soma contact (arrow). H/E stain (i) x600 (ii) x1250.



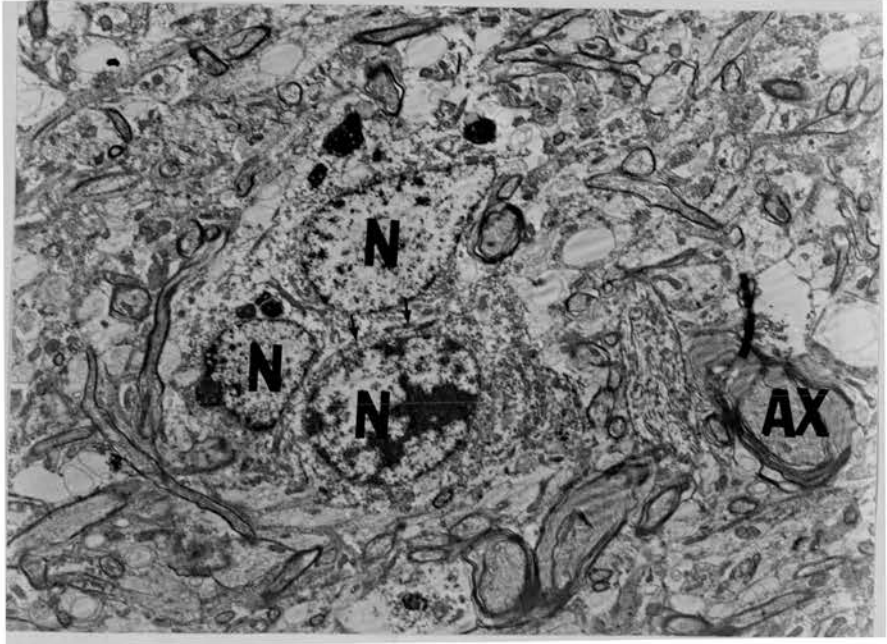


Figure 14. Electron micrograph of a cluster of 3 mesencephalic neurons of monkey showing soma-soma contact of cell membranes (arrows). Nucleus (N) Axons (Ax) x2900.

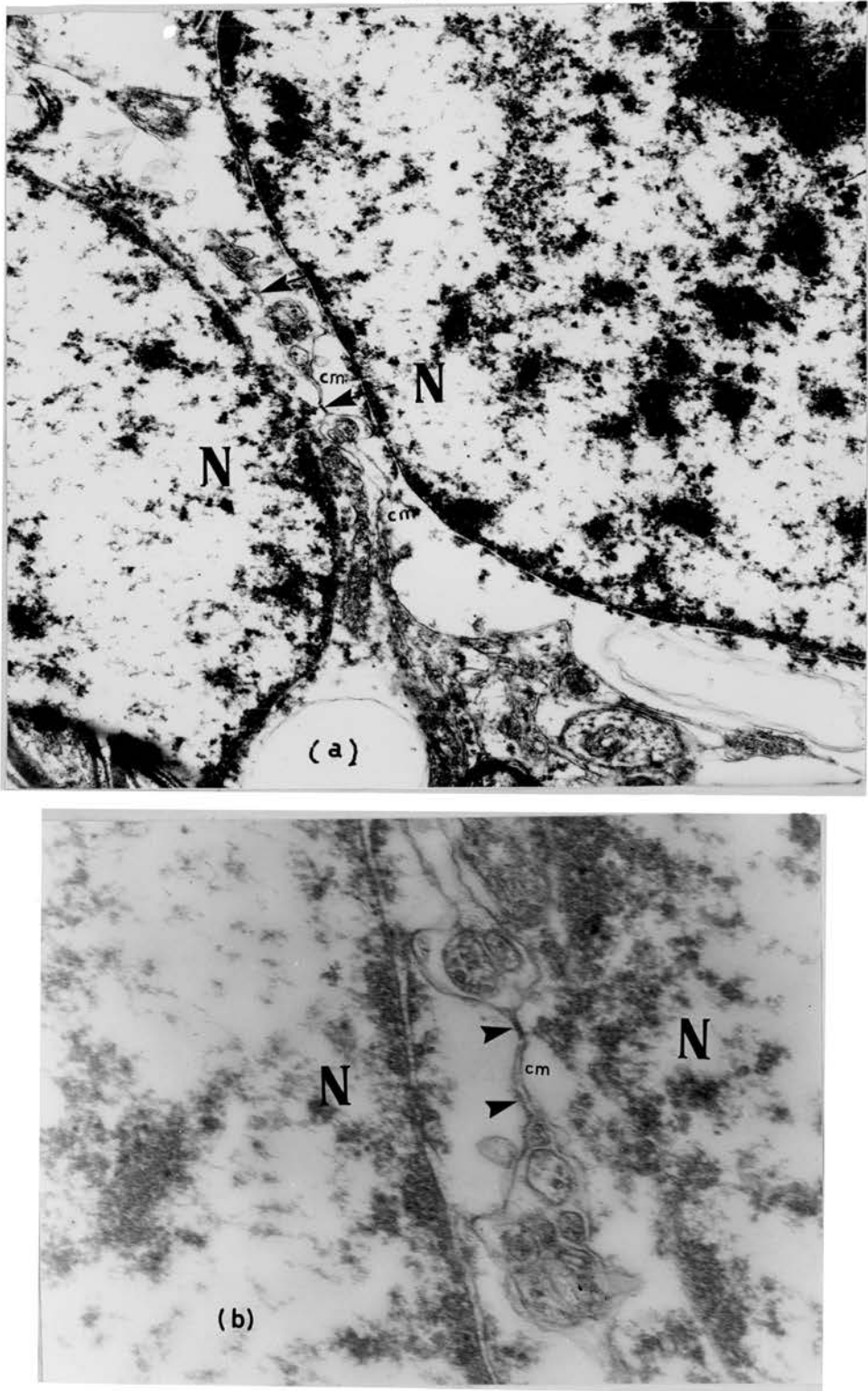


Figure 15. Electron micrographs of sections of two mesencephalic neurons of monkey (a) in soma-soma contact of cell membranes (arrows)  $\times 23000$ . (b) shows the area of cell membrane contact where maculae adherens gap junctional contact is apparent (arrowheads)  $\times 43000$ . Nucleus (N), Cell membrane (cm).

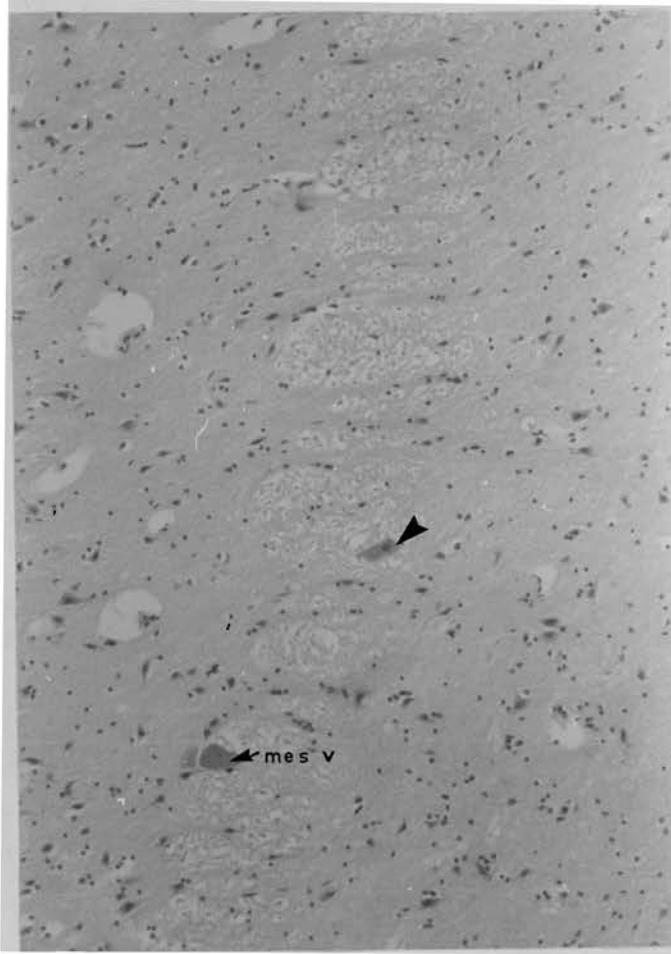
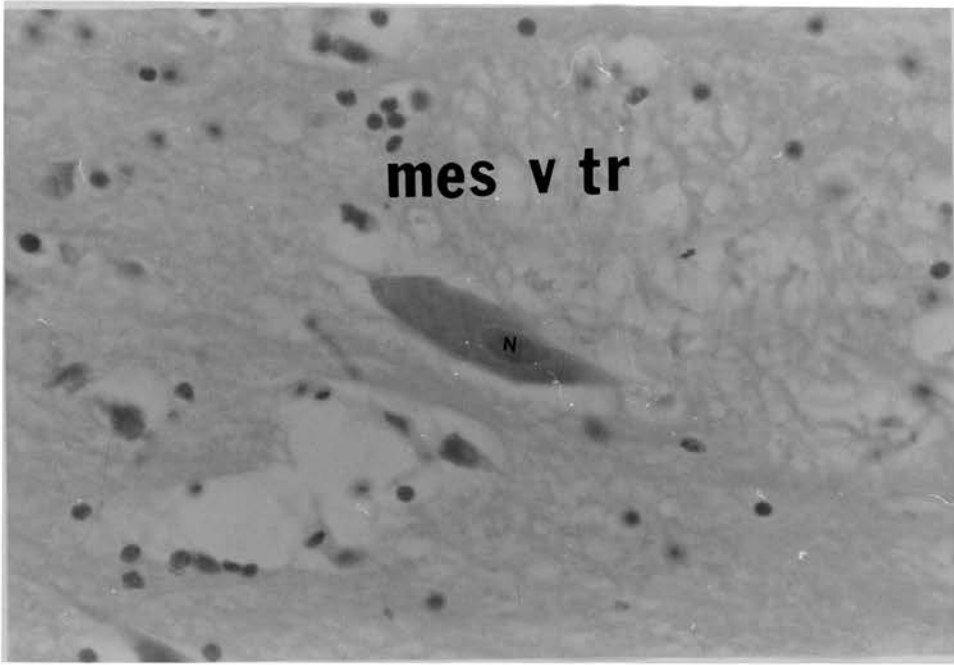
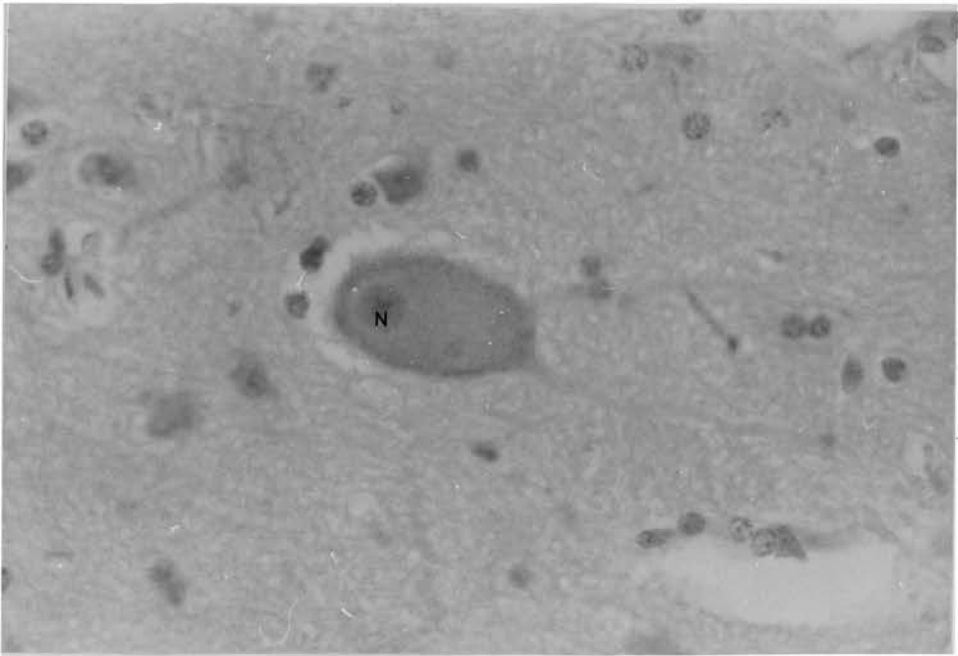


Figure 16(a). Photomicrograph of paraffin wax section (10 $\mu$ m) of the baboon midbrain (T.S.) at the caudal level of the inferior colliculi showing sparse mesencephalic neurons (Mes V) and an intrafascicular neuron (arrowhead) H/E stain. x125.

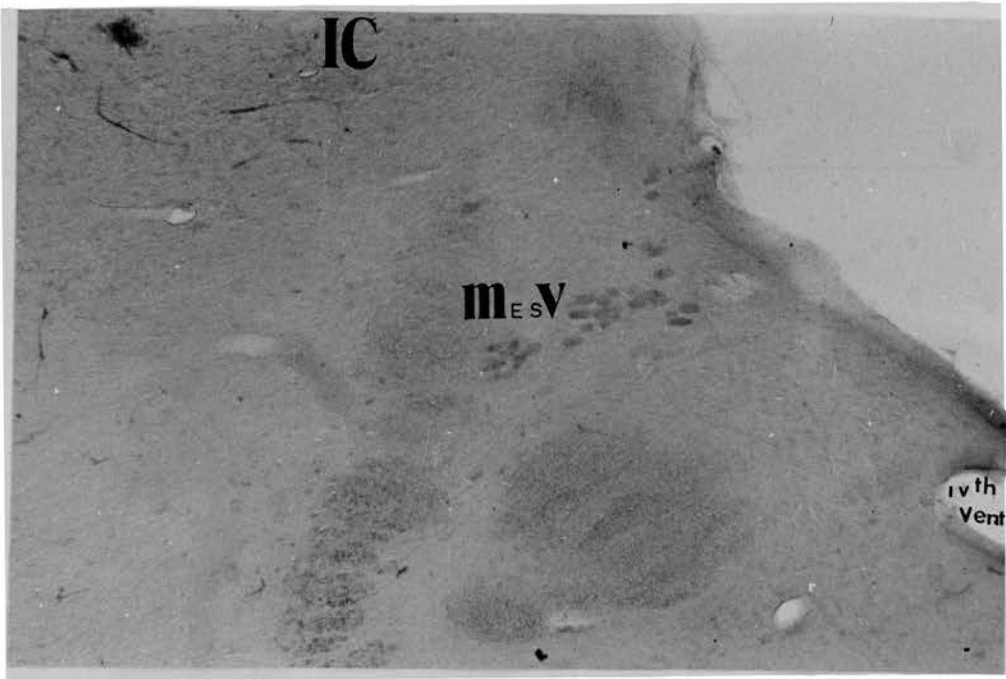


(i)

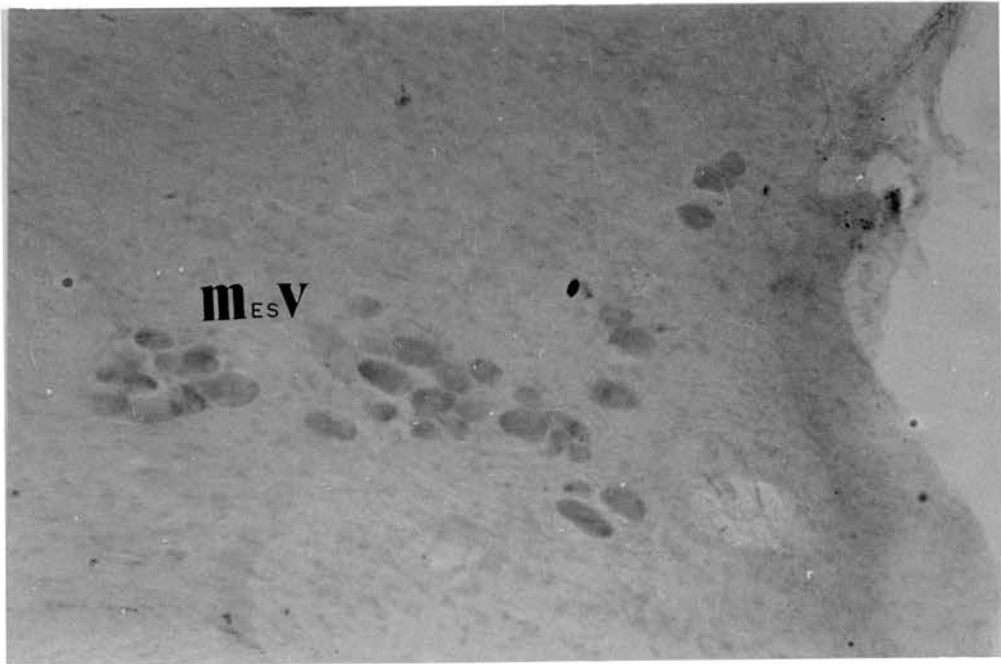


(ii)

Figure 16(b). Photomicrographs of (i) An intrafascicular fusiform mesencephalic neuron and (ii) oval bipolar mesencephalic neuron in paraffin wax sections (10 $\mu$ m) of baboon midbrain. H/E stain x300 Nucleus (N). Mesencephalic tract (Mes V-tr).



(i)



(ii)

Figure 17(a). Photomicrographs of transverse frozen section (80 $\mu$ ) of rostral aspect of pons at the junction of the inferior colliculi (IC) of vervet monkey showing large clusters of mesencephalic neurons (Mes V) on the dorso-lateral aspect of the fourth ventricle (IVth Vent.) Neutral red stain (i)  $\times 50$  (ii)  $\times 125$ .



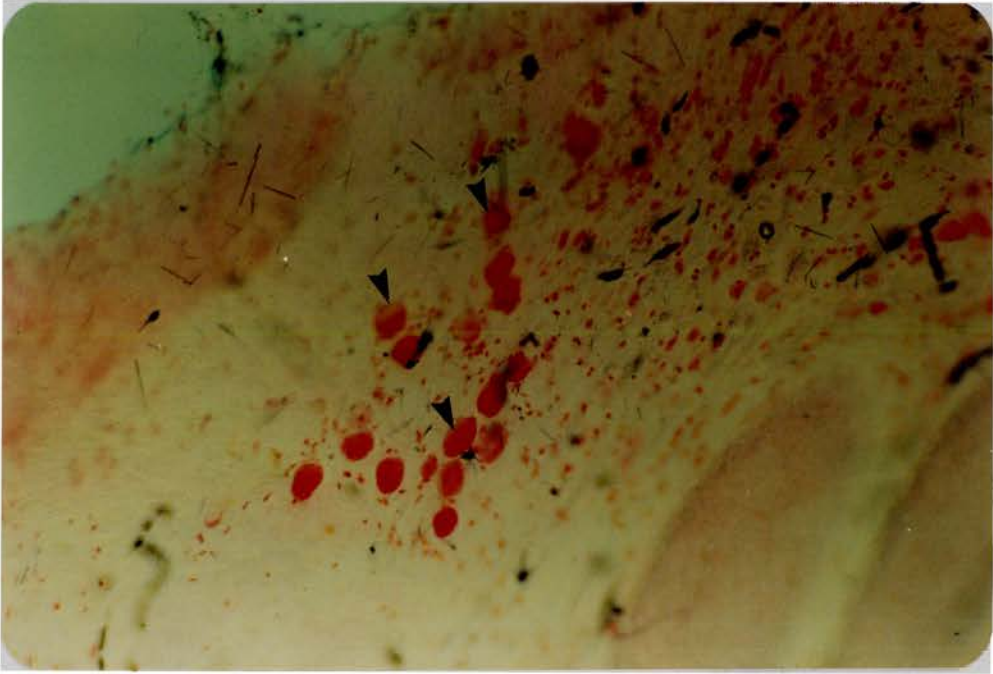


Figure 17(b). Photomicrograph of transverse frozen section (80µm) of rostral aspect of pons of vervet monkey showing an aggregation of large numbers of mesencephalic neurons (◄), Neutral red stain. x125.

3.14 The caudal limit of the mesencephalic nucleus in the pons was found to be indistinct in both the vervet monkey and <sup>the</sup> olive baboon. In this region the mesencephalic neurons are located close to the cells of locus coeruleus and the distinction between the mesencephalic neurons and the cells of locus coeruleus was often not clear (Fig.18). The trigeminal mesencephalic neurons show some histological similarities to the cells of locus coeruleus (Figs.18, 19). Generally, the mesencephalic cells were densely staining unipolar cells or small multipolar cells located medial to the mesencephalic tract on the dorso-lateral aspect of the locus coeruleus (Fig. 18, 19). The cells of the locus coeruleus are dark or pale staining multipolar cells with a single nucleus and nucleolus (Fig.19). These cells are medium sized, round or oval in shape. These cells of locus coeruleus often contain neuromelanin, a brown pigment. There was lack of consistency in the appearance of the brown granules in the cells of locus coeruleus in the frozen and paraffin wax sections of the pons of various monkeys and baboons. Brown granules were distinctly observed in some and not <sup>in</sup> other animals.

3.15 An aggregation of bipolar and multipolar neurons was observed at the junction of locus coeruleus and the motor nucleus of trigeminal nerve. <sup>(Figs 18,19).</sup> The cells were either lightly or densely staining, small and elongated. <sup>(Fig.20)</sup> The

cells extended along the outer margin of the mesencephalic tract across from the ventro-lateral tip of the locus coeruleus and formed a group of neurons adjacent to the motor nuclei of the trigeminal nerve. Some neurons in the group contained brown pigment similar to locus coeruleus cells and were intermingled with the non-pigmented cells. It is suggested that the aggregation of these cells may constitute the supratrigeminal nucleus which is thought to be a group of interneurons (Fig. 18, 19, 20).

3.16 Lipofuscin pigment was also observed in some of the cells of the mesencephalic nucleus of trigeminal nerve as clumps of yellow pigment. The pigment was concentrated mainly on one pole of the neuron. Lipofuscin was observed in most of the cells within the brainstem in two adult and two young vervet monkeys and three adult baboons.

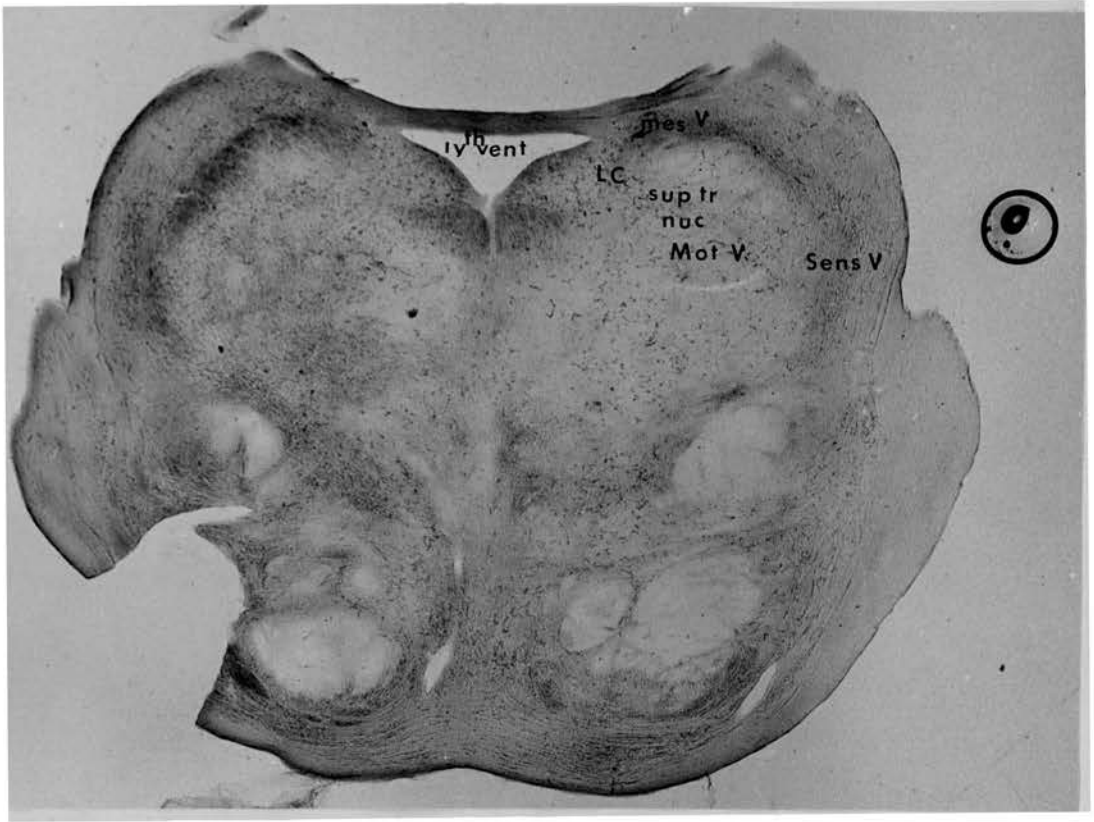
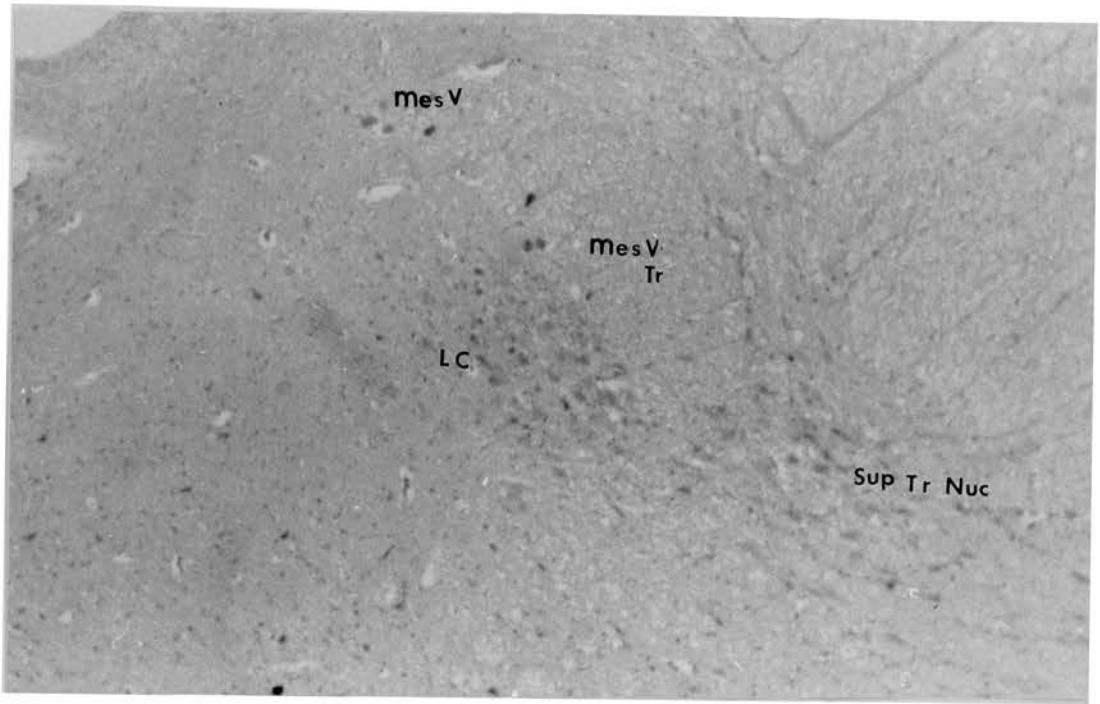
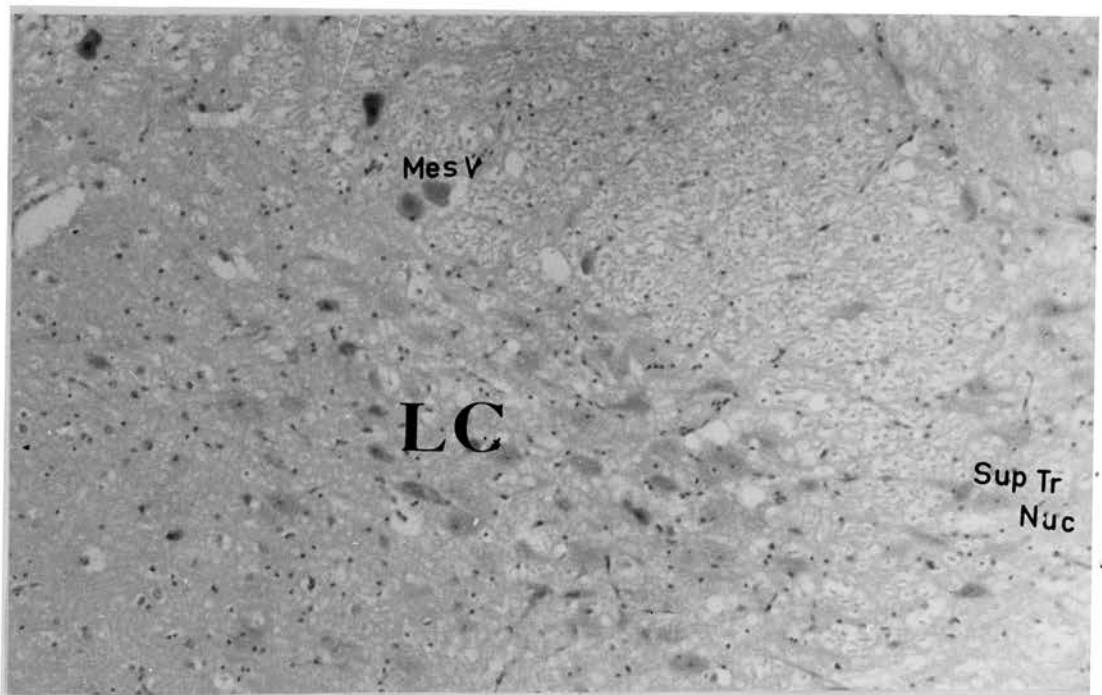


Figure 18. Photomicrograph of transverse frozen section profile (80µm) of rostral pons showing the position of mesencephalic neurons (Mes V), Locus coeruleus (LC) the supratrigeminal nucleus (Sup. Tr. Nuc.), Motor nucleus of trigeminal (Mot. V.) and sensory nucleus of trigeminal (Sens. V). Fourth Ventricle (IV<sup>th</sup> Vent) X 10. Neutral red stain.



(i)



(ii)

Figure 19. Photomicrographs of paraffin-wax section (10 $\mu$ m) of pons (T.S.) of baboon showing the caudal extent of the mesencephalic nucleus. Mesencephalic neurons (Mes V), Mesencephalic tract (Mes V-Tr.), Locus coeruleus (LC), (Sup Tr Nuc) Supratrigeminal nucleus. H/E stain. (i) x50 (ii) x125.

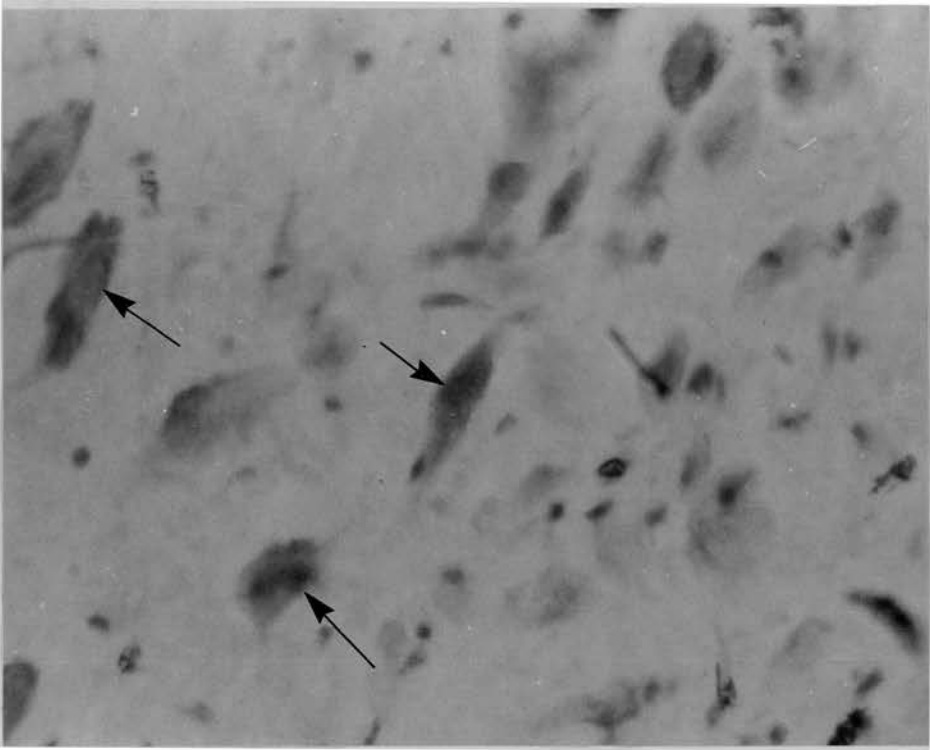


Figure 20. Photomicrograph of frozen section (80 $\mu$ m) of monkey pons showing the neurons (arrows) in the region of the supratrigeminal nucleus. Neutral red stain. x300.

B. Quantitative Analysis of the Mesencephalic Nucleus of the Trigeminal Nerve in the Vervet Monkey and Olive Baboon

3.17. The quantitative analysis of the distribution of the mesencephalic neurons along the rostro-caudal extent of the nucleus in the vervet monkey and olive baboon revealed that regional variations exist in the numerical density of cells along the rostro-caudal extent of the nucleus in the vervet monkey and olive baboon. The numerical density of cells from the anterior limit of the nucleus to the inferior colliculi showed that there was a regional aggregation of cells around the mid-superior collicular and mid-inferior collicular levels. The relative density of cells diminished at the caudal limit of the inferior colliculi and increased in the rostral pontine region. The highest number of cells was found in the mid-superior collicular region with a general decrease in the number of cells towards the caudal region of the nucleus (Table 3, Fig. 21).

3.18 From the bilateral mesencephalic cell counts of serial paraffin-wax sections of 6 monkeys (A-F) and 4 baboons (1-4), the total number of cells was calculated for each consecutive 0.5 mm of the brainstem from the rostral to caudal extent. These values were obtained from the serial sections of brainstem of each of the monkeys and baboons. Mean values, S.D. and S.E. for the



0.5 mm of the brainstem of 6 vervet monkeys and 4 olive baboons were obtained as shown in table 3a and 3b respectively. (One of the monkeys, (G) brainstem, which had been obliquely transected was omitted). The S.D. of the mean seen in Table 3 suggests that there may be large variations in the number of cells in 0.5 mm of the brainstem at any one level, between the brainstems of the various monkeys and baboons respectively. The variations in numbers may be as a result of actual variation in the density of cells at any one level along the rostro-caudal extent from one animal to the next. It may however, be due to the differences in the level of each 0.5 mm of the brainstem for which the total number of cells was calculated, since the data was obtained from the serial sections of varying thickness.

3.19 The numerical differences of about 2 to 10 cells between the right and left sides were noted in many sections at any one level along the rostro-caudal extent of the brainstem. The overall difference in the total count of the right and left sides was not marked. In two monkeys the total count was 557 and 778 on the left side and 514 and 867 on the right side respectively. In one of the baboons the total cell count on the right side was 664 and left side was 648. This difference in the cell counts on the right and left side of a section may be due to variation in the position of cell aggregation on the right and left side along the rostro-caudal extent of the



nucleus, and/or the obliquity of the sectioning in the coronal plane.

3.20 The total cell counts of mesencephalic nucleus obtained from the actual counts of cells from all the collected serial sections of the various brainstems are shown in Tables 3a and 3b for the monkeys and baboons respectively. Variations in the values of the total counts (T count) were observed for the brainstems of the monkeys and baboons respectively. The range of total counts was 810 - 1821 with a mean of  $1341 \pm 380$  for the monkey and 1312 - 2063 with mean of  $1620 \pm 366$  for the baboon.

3.21 The quantitative distribution of the mesencephalic neurons along the rostro-caudal extent of the mesencephalic nucleus in the vervet monkey and olive baboon is shown in Fig. 21. Each 0.5mm bin represents the mean values of bilateral counts. The vertical bars represents the S.E. of the means of bilateral counts. The rostro-caudal extent of the mesencephalic nucleus was about 11 mm in the monkey and about 13 mm in the baboon. The superior colliculi extended about 4 mm, inferior colliculi 3.5 mm and pons 3.5 mm in the monkey. In the baboon, the superior colliculi extended about 4.5 mm, inferior colliculi 4 mm and pons 4.5 mm. The quantitative distribution of the mesencephalic neurons in Fig. 21 shows that the number of mesencephalic neurons

increase to the mid-superior collicular level from the anterior limit of the nucleus. The cell density increases to a peak up to the mid superior collicular level, and then gradually begins to diminish caudally. At the mid-inferior collicular level, there is an increase in the number of cells. The number of cells then markedly decreases towards the caudal-inferior collicular region and rises again in the rostral pontine region. In the caudal part of the nucleus, the cell numbers gradually decrease .

3.22 The microscopic observations of the mesencephalic neurons showed that there were some variations in the size and shape of the mesencephalic neurons in the vervet monkey and olive baboon. Majority of the cells were medium to large sized, round or oval, found along the rostro-caudal extent of the midbrain and pons. There were some bipolar or fusiform and multipolar neurons mainly in the caudal part of the mesencephalic nucleus. The data on the diameters of mesencephalic neuron was obtained from measurements made on 100 neurons in the paraffin wax sections of the brainstem of the vervet monkey and olive baboon respectively. In the mesencephalic nucleus of the monkey, the range of diameter of the neurons was 15 - 60 $\mu$ m, mean  $33 \pm 6\mu$ m and in the baboon, the range was 18 - 75 $\mu$ m, with a mean of  $39 \pm 8\mu$ m. Sixty percent of the neurons were large sized with a range of about 30 - 60 $\mu$ m, 35% of the cells were medium

sized with a range of about 15 - 30 $\mu$ m. 3% of the neurons were bipolar or fusiform with the maximum diameter along the long axis, of 60 - 70 $\mu$ m and minimum diameter of 20 $\mu$ m. About 2% of the neurons were multipolar with the soma diameter of 20 - 30 $\mu$ m. The measurements of cells obtained from the paraffin wax sections usually require correction for shrinkage of the tissue due to fixation, dehydration and paraffin-wax embedding. (Blinkov and Glezer, 1968). The amount of shrinkage estimated for brainstem tissue was found to <sup>be</sup> about 15%. The corrected mean diameter of the monkey mesencephalic neuron was about 38 $\mu$ m and of the baboon 45 $\mu$ m. The difference between the diameter of the mesencephalic cells of the monkey and baboon is significant ( $p < 0.01$ ).

3.23 The mean values, S.D. of the volume proportion  $V_V$ , number per unit area ( $N_A$ ) and the numerical density ( $N_V$  of the mesencephalic neurons obtained from stereological analysis of serial, stratified paraffin wax sections of 7 vervet monkeys and 4 baboons are shown in Tables 4a and 4b respectively. The data was obtained from the serial sections of the brainstem of six monkeys A - F (see Table 3a) and 4 baboons, 1 - 4 (see Table 3b). In addition, the brainstem of monkey G which had been transected obliquely was also analysed. The mean values presented in Table 4 were obtained from the analysis of 10 - 20 equidistantly spaced serial sections. For each of the section of the brainstem that was analysed,  $V_V$

$N_A$  and  $N_V$  were obtained and the mean values were calculated from data of all sections analysed from one brainstem. The variation in the volume proportions and numerical densities of the mesencephalic neurons along the rostro - caudal extent of the nucleus is similar to the variation in numerical distribution of the neurons shown in Fig 21. In the monkey, the mean fraction  $V_V$  of mesencephalic neurons is found to be 0.0008 and in the baboon it was 0.00065 showing that mesencephalic nucleus consists of 0.08% of the brainstem in the monkey and 0.065% in the baboon. The number per unit area of the mesencephalic neurons in the various monkeys and baboons is very similar in that about 0.15 neurons are found in one  $\text{mm}^2$  of the brainstem of monkey and 0.12 in the baboon. The numerical density  $N_V$  of the mesencephalic neurons, calculated using the formulae of Weibel and Gomez (1962),  $N_V - a$  and that of Weibel (1979)  $N_V - b$  are shown in Table 4. The value of the diameter (D), of the mesencephalic neurons used in the calculation of  $N_V - b$  is the corrected mean diameter, (38 $\mu\text{m}$  for the monkey and 45 $\mu\text{m}$  for the baboon). There are 2 - 3 neurons per  $\text{mm}^3$  of the brainstem in the monkey while in the baboon there are about 2 neurons in  $\text{mm}^3$  of the brainstem. The volume of brainstem of two monkeys (A and C) and two baboons (2 and 4) were measured prior to sectioning. The monkey brainstem volume from the rostral level of the superior colliculi to the anterior part of pons,

approximately along the extent of mesencephalic nucleus was 1.14ml for (A) and 1.4ml for (C). In the baboon it was 1.92ml for (2) and 1.6ml for (4), the latter being the brainstem of a young baboon. The estimated total number of cells was calculated using the value of  $N_V$  - a

3.24 The estimated value of the total number of neurons exceeds that obtained from the actual cell counts in the same brainstem (Table 4). There is variation in the values of the total cell counts obtained from different monkeys and baboons. The coefficient of variation of the mean is about 22-26%. Student's t-test showed that the differences between the mean values of the total bilateral mesencephalic cell counts in monkey ( $1379 \pm 362$ ,  $n=7$ ) and the baboon  $1620 \pm 366$ ,  $n=4$  were significant ( $p<0.1$ ).

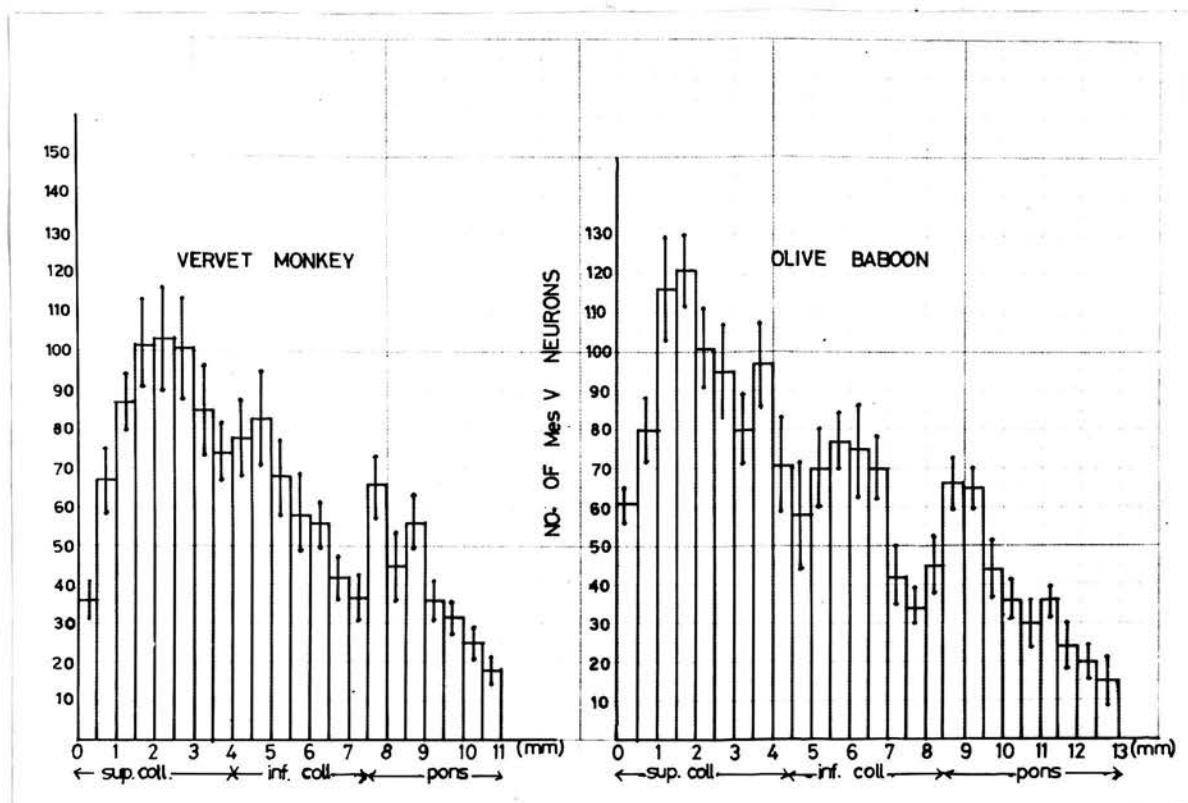


Figure 21. Histogram Showing the distribution of the mesencephalic neurons in each 0.5mm of the brainstem along the rostro-caudal extent of the mesencephalic nucleus in the vervet monkey and olive baboon.

Table 3a showing the means SD and SE of bilateral Mes V cell counts, in each 0.5mm of the brainstem from rostral to the caudal extent of the Mes V nucleus in the vervet monkey.

Monkey	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	Total
A 10µm every 5th section	38	77	103	131	121	100	96	76	82	94	88	60	54	44	52	75	73	41	43	27	16	13	1504
B 20µm all sections	51	89	93	91	130	124	96	82	91	119	78	76	68	52	48	83	68	54	46	44	34	27	1645
C 7µm every 5th section	46	86	112	136	144	151	122	91	108	105	98	92	70	54	38	76	64	78	52	40	32	26	1821
D 7µm every 10th section	20	48	72	97	73	68	55	43	32	42	31	32	38	20	22	33	36	28	20	-	-	-	810
E 7µm every 10th section	26	47	65	68	78	76	44	86	74	78	50	42	54	44	30	58	42	24	24	22	25	14	1071
F 7µm every 10th section	33	54	78	86	70	88	95	65	82	68	61	46	53	38	31	73	53	41	33	28	19	12	1197
Mean	36	67	37	102	103	101	85	74	78	83	68	58	56	42	37	66	45	56	36	32	25	18	1341
S.D	12	19	18	27	32	31	29	18	15	29	25	22	12	12	11	18	14	20	13	9	8	7	380
S.E	5	8	7	11	13	13	12	7	10	12	10	9	5	5	5	7	6	8	5	4	4	3	155

Table 3b showing the means, SD and SE of bilateral Mes V cell counts, in each 0.5mm of the brainstem from rostral to the caudal extent of the Mes V nucleus in the olive baboon.

Baboon	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	Total
1. 10µm every 10th section	56	72	98	107	81	84	74	70	42	30	60	64	58	50	38	27	40	60	50	36	31	25	29	15	20	-	1312
2. 10µm every 5th section	64	91	122	134	109	95	83	106	86	78	65	91	92	85	61	38	47	62	71	34	37	28	39	20	27	14	1779
3. 7µm every 10th section.	54	60	92	104	90	73	59	88	61	42	58	65	52	63	40	28	32	56	65	42	26	20	32	18	10	6	1326
4. 10µm every 5th section	70	95	150	139	125	129	102	123	95	83	98	87	99	80	31	43	62	84	73	62	50	48	44	42	23	26	2063
Mean	61	80	116	121	101	95	80	97	71	58	70	77	75	70	42	34	45	66	65	44	36	30	36	24	20	15	1620
SD	7	16	26	18	20	24	18	23	24	26	19	14	24	16	13	8	13	12	10	13	10	12	17	12	7	10	366
SE	4	8	13	9	10	12	9	11	12	13	10	7	12	8	7	4	7	6	5	7	5	6	3	6	4	6	183

Table 4 showing the mean values and SD of volume proportions ( $V_V$ ) number per unit area ( $N_A$ ) and the numerical density ( $N_V$ ) of the Mes V neurons in the brainstem of vervet monkey (4a) and olive baboon (4b). The estimated total Mes V cell counts ( $N_{T(est)}$ ) from the volume of the brainstem and the (T count), total bilateral counts are also shown.

Table 4a - Vervet Monkeys

Monkey	Section Thickness	$V_{Vmes}$	$N_{A/mm^2}$	(a) $N_{V/mm^3}$	(b) $N_{V/mm^3}$	$N_{T(est)}$	T Count
A	10 $\mu m$	0.0008	0.142	0.190	3.0	2160	1504
B	20 $\mu m$	0.0007	0.140	1.85	2.4	-	1645
C	7 $\mu m$	0.0008	0.143	1.91	3.2	2674	1821
D	7 $\mu m$	0.001	0.153	1.89	3.4	-	810
E	7 $\mu m$	0.0008	0.160	2.00	3.6	-	1071
F	7 $\mu m$	0.0006	0.141	1.64	3.1	-	1197
G	7 $\mu m$	0.0008	0.160	2.00	3.6	-	1608
MEAN	-	0.0008	0.148	1.88	3.19	-	1379
SD	-	0.0001	0.0089	0.42	0.42	-	362

Table 4b - Olive Baboon

Monkey	Section Thickness	$V_{Vmes}$	$N_{A/mm^2}$	(a) $N_{V/mm^3}$	(b) $N_{V/mm^3}$	$N_{T(est)}$	T Count
1	10 $\mu m$	0.0007	0.115	1.50	2.09	-	1312
2	10 $\mu m$	0.00055	0.01	1.42	1.84	2730	1779
3	7 $\mu m$	0.0006	0.117	1.70	2.25	-	1326
4	10 $\mu m$	0.0007	0.128	1.76	2.23	2816	2063
MEAN	-	0.00064	0.115	1.60	2.13	-	1620
SD	-	0.00076	0.01	0.16	0.02	-	366

$$N_{Va} = \frac{(N_A)^{3/2}}{V_{Vmes}}$$

$$N_{Vb} = \frac{N_A}{*D + t}$$

\*D = mean diameter of neurons  
monkey 38  $\mu m$  and baboon 45  $\mu m$   
t = section thickness



C. General Distribution Of The HRP Labelled  
Mesencephalic Neurons Of The Trigeminal Nerve In The  
Vervet Monkey And Olive Baboon.

3.25. HRP labelled neurons in the mesencephalic nucleus of the trigeminal nerve were observed on the ipsilateral side of the mainly caudal part of the nucleus following HRP injections into the periodontal ligament and gingivae of incisors, canines and molars in the vervet monkey and olive baboon. No contralateral HRP labelled mesencephalic neurons were observed in animals injected with the HRP in the teeth on the unilateral side. No HRP labelled neurons were observed following injection into the tooth pulp.

3.26. The HRP reaction product in the mesencephalic neurons appeared as fine blue-black granules when observed in bright field microscopy soon after histochemical reaction with TMB and subsequent counterstaining with neutral red within 24-48 hours (Figs. 22,23). The colour of the blue-black HRP granules showed up as good contrast against the lightly pink unlabelled cells and the pale background (Figs 23 a, b). The HRP granules were easily visible if the cell was densely filled (Fig. 22). When the cell was very densely filled, the grains were not easily discerned, though the periphery of the neurons was well defined and

few fine grains could be seen in the axon hillock region and in the cell process (Figs. 23, 24). When the neuron was very lightly filled, the HRP granules were sparsely distributed within the perikaryon (Fig. 25). Visibility of very lightly filled cells was often difficult when the sections were about 100um. The HRP granules appeared dark blue / brown if observed after some period of time following HRP histochemical reaction and counterstaining with neutral red. Some of the HRP labelled cells appeared to contain brown crystalline or clumpy products' which were sometimes difficult to differentiate from the large brown artifacts (Fig.26). The position of the neuron and other characteristic features of mesencephalic neuron were then taken into consideration to determine if these were HRP labelled cells or artifacts.

3.27 Stability of HRP reaction product was variable. The HRP reaction product generally tended to fade away with time and prolonged exposure to strong day/light. In some cases, the reaction product faded very fast, within two to three weeks in spite of storage of the slides in boxes placed in dark cupboards. The rate of fading of HRP reaction was quite variable such that in a section, dehydrated with isopropyl alcohol, some of the labelled cells remained with stable clearly visible HRP granules for up to 2-3 years while others faded away within 1-2

months. The HRP reaction product was very prominent and intense in sections dehydrated with ethanol, but faded away very quickly within 2-4 weeks.

3.28 The red blood corpuscles have endogenous peroxidase, which following oxidation with  $H_2O_2$  and TMB histochemical reaction results in a blue-black product. The blood vessels which were not completely cleared of blood during perfusion appeared as many blue-black strands within the section (Figs. 23, 24). Generally, blood vessels and red blood corpuscles were easily recognized. However, the "background staining" sometimes made observations of the HRP labelled neurons difficult, and at times obscured the labelled cells (Figs. 22, 24, 28, 29). The blood vessels were most profuse in the tectum of the midbrain, especially at the level of the inferior colliculi (Figs. 22, 24). In the pons the blood vessels were visible in the periventricular gray matter (Figs. 27, 28).

3.29. In addition, there were other artifacts in the background. These were mainly needle-like processes or dispersed crystalline structures. These needle-like processes<sup>es</sup> were seen as thin sharp structures (Fig. 30). A very dense fine brown granular sediment was noted spread over large areas of the section in some instances. This was noted in a few TMB reacted sections that had been

bathed in post-reaction medium for less than one hour. Such deposits also obscured the visibility of structures and cells in the sections.

3.30. Apart from the HRP reaction product, there was another kind of pigment noted in some of the mesencephalic neurons. In two baboons (No. 864 and 33) and two monkeys (No. 1 and 7) brainstem frozen sections, yellow diffuse clumps of pigment <sup>were</sup> present in almost all the cells and appeared as lipofuscin aggregated at one pole of the neuron. There was no apparent relation to age of the animal. Baboon (33) was old while No. 864 was young. Another type of brown pigment, neuromelanin is also thought to be present occasionally in some mesencephalic cells. If present, this was not easily recognized and may have been obscured by the background staining or identified as lightly labelled cells.

3.31. The distribution of the HRP labelled mesencephalic neurons from the periodontal ligament and gingivae of the various teeth types was similar in the vervet monkey and olive baboon. HRP labelled mesencephalic neurons were located mainly in the caudal part of the nucleus at the level of the inferior colliculi and pons (Figs. 22, 24, 27, 28, 29). Few labelled neurons were observed at the level of the superior colliculi.

3.32 The HRP labelled neurons were mainly large and medium-sized spherical or oval neurons. These neurons were observed often as a labelled neuron adjacent to an unlabelled neuron (Fig.22), or as a single isolated labelled neuron (Fig 23b). Occasionally a large-sized labelled neuron was observed close to many unlabelled neurons (Fig. 23a). At the level of the inferior colliculi, a fusiform labelled neuron was observed within the mesencephalic tract (Fig.29). Two labelled neurons were sometimes observed adjacent to one another particularly in the region of the pons (Fig.28).

3.33. In the caudal region of the mesencephalic nucleus, small, generally faintly labelled cells were observed ipsilaterally at the level of the nucleus locus coeruleus. The labelling in these neurons appeared to be brown and sparsely distributed within the cell body (Fig.30). Some of these smaller labelled mesencephalic cells appeared to be multipolar and were somewhat similar to the cells of locus coeruleus (Fig.30).

3.34 More caudally in pons, at the level of the motor nucleus of trigeminal nerve, some "faintly labelled" cells were observed bilaterally following HRP injection to the periodontal ligaments of various teeth types on the ipsilateral side (Fig.31). These faintly labelled cells were located mainly in the region extending from

the ventrolateral aspect of locus coeruleus cells (Fig.32). Adjacent section of pons not processed for TMB reaction product was observed to determine if the "faintly labelled" cells were pigmented locus coeruleus cells. In the processed sections the "labelled cells" were more prominent and more in numbers than some locus coeruleus cells showing sparse indistinct brown granules. Although difficulties were encountered in deciding the precise location of these neurons, a careful analysis of many sections showed that majority of the "labelled cell" were close to and/or occurred in the region of the supratrigeminal nucleus.

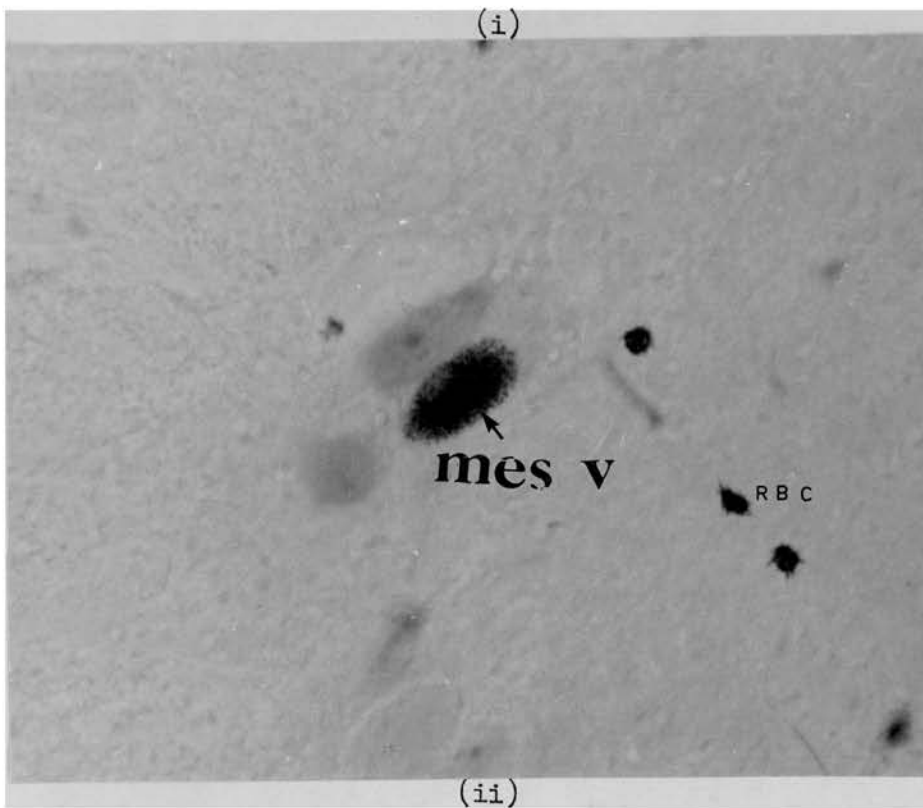
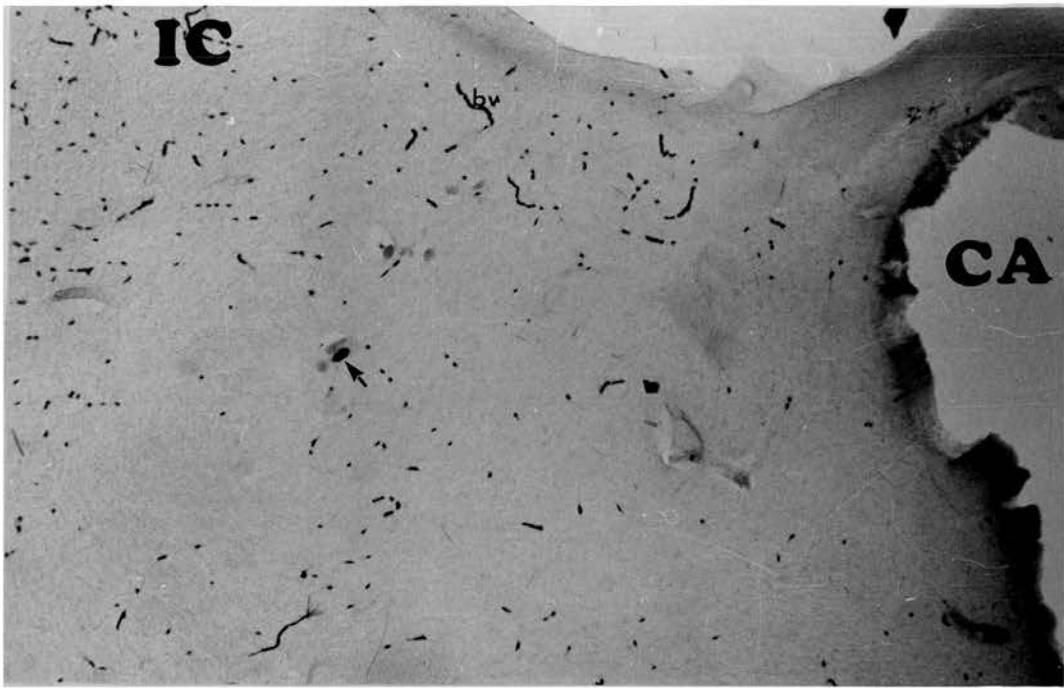
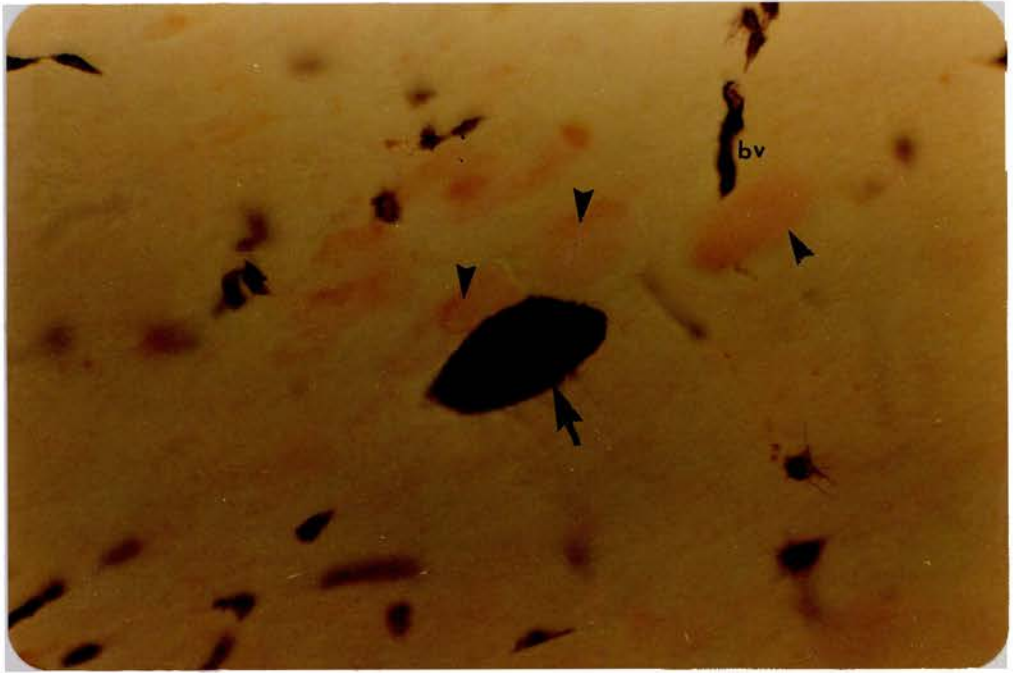
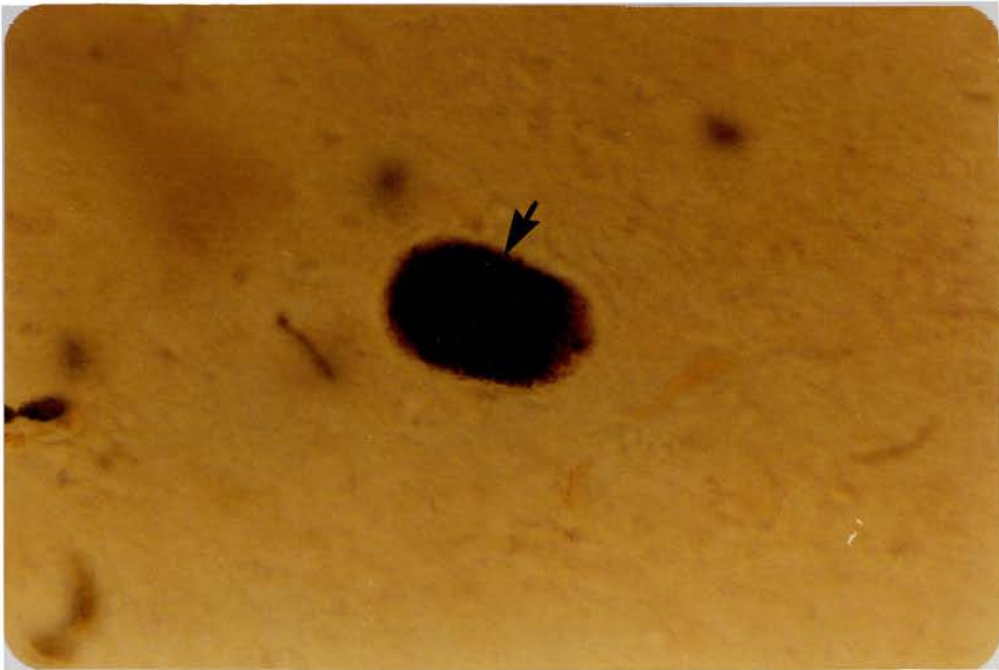


Figure 22. Photomicrographs of frozen section (50 $\mu$ m) of midbrain of baboon (T.S.) at the level of inferior colliculi (IC) showing an HRP labelled mesencephalic neuron (arrow - Mes V) adjacent to an unlabelled neuron. Cerebral aqueduct (CA). Neutral red stain. (i)  $\times 50$  (ii)  $\times 125$ . Blood vessel (bv), Rcd blood corpuscle (RBC).





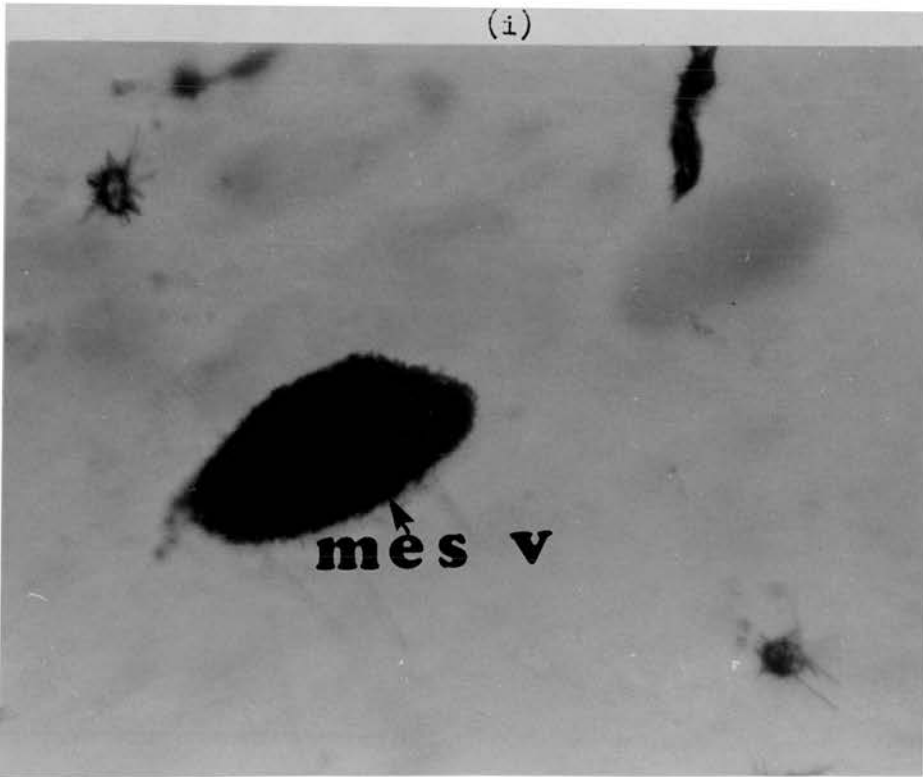
(a)



(b)

Figure 23. Photomicrographs of frozen sections midbrain (50µm) of monkey (T.S.) showing densely labelled mesencephalic neurons (a) large HRP labelled neuron (arrow) adjacent to unlabelled neurons (arrowheads) in a cluster x400. (b) A single HRP labelled neuron x500. Neutral red stain. Blood vessel (bv).





(ii)

Figure 24. Photomicrographs of frozen section (50 $\mu$ m) of midbrain of the monkey, (T.S.) at the level of the caudal aspect of inferior colliculi (IC) showing a densely HRP labelled mesencephalic neuron (arrow - Mes V), cerebral aqueduct (CA). Neutral red stain. (i) x50 (ii) x600.

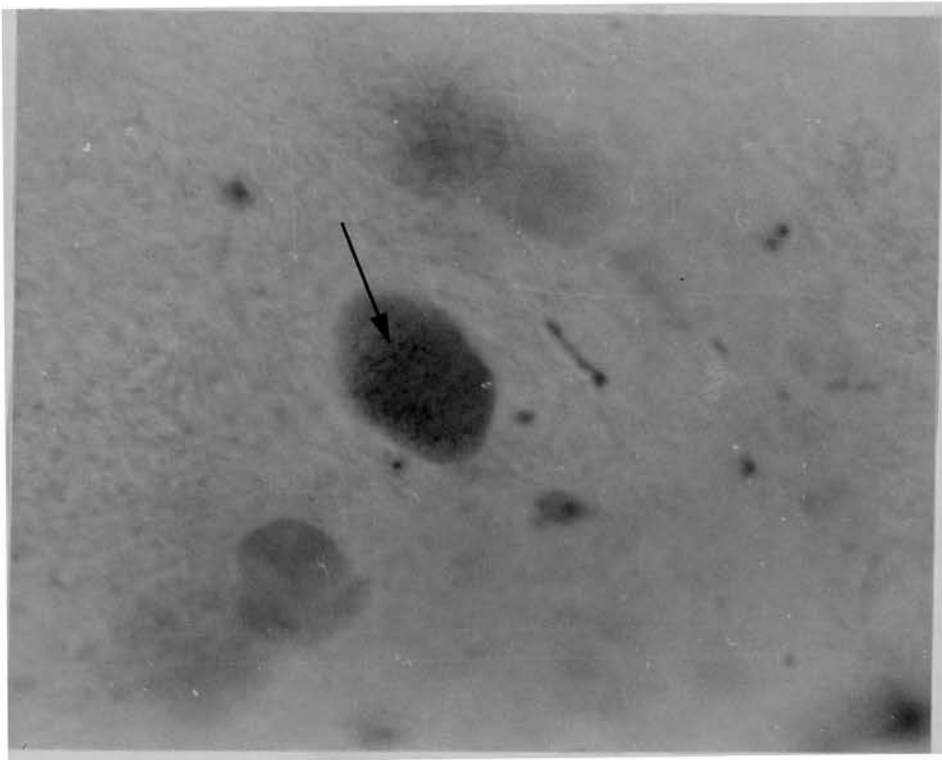


Figure 25. Photomicrograph of frozen section of midbrain of monkey (T.S.) showing a lightly HRP labelled mesencephalic neuron (arrow). Neutral red stain. x600.

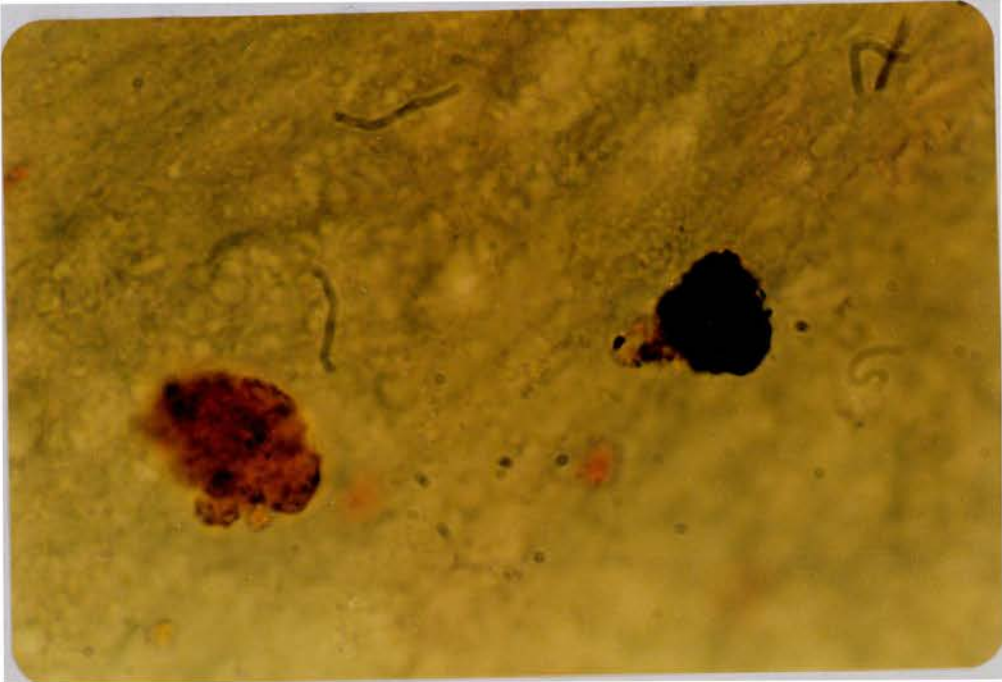
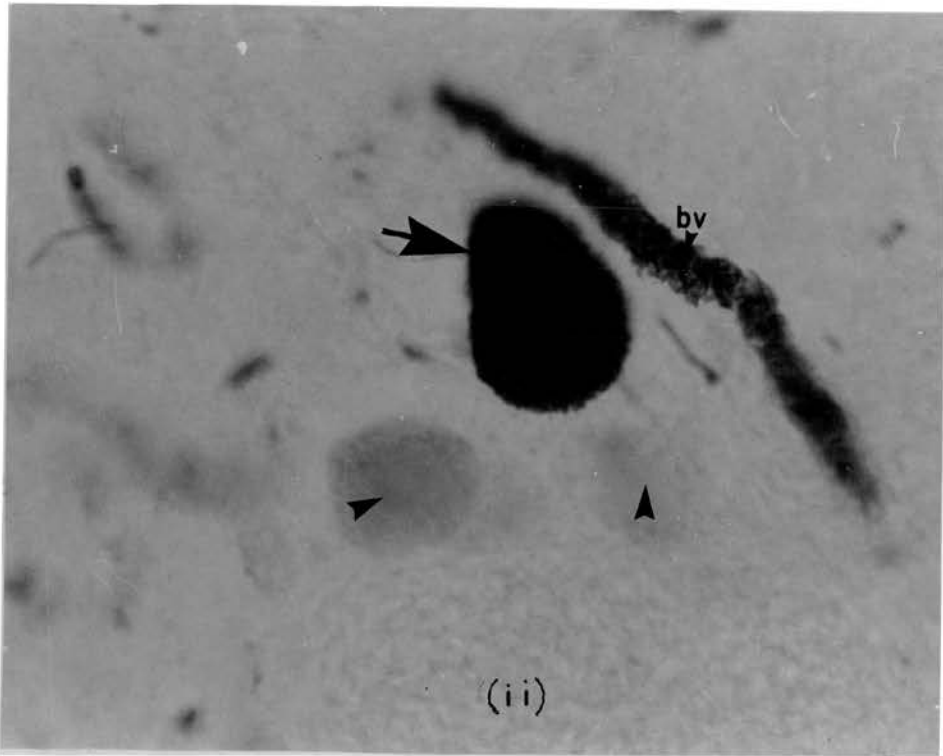


Figure 26. Photomicrograph of frozen section of midbrain of monkey (T.S.) showing a densely labelled mesencephalic neuron on the right. The "HRP granules" in the cell on the left appear as coarse and crystalline similar to some artifacts. Neutral red stain. x500.



(i)



(ii)

Figure 27. Photomicrographs of frozen section of pons of monkey (T.S.) showing a large single HRP labelled mesencephalic neuron (arrow) near some unlabelled mesencephalic neurons. (arrowhead) Neutral red stain (i) x50 (ii) x600. Blood vessel (bv), Fourth ventricle (iv<sup>th</sup> vent).



Figure 28. Photomicrograph of frozen section of pons (T.S.) of monkey showing two adjacent HRP labelled mesencephalic neurons (arrow). Neutral red stain. x40.

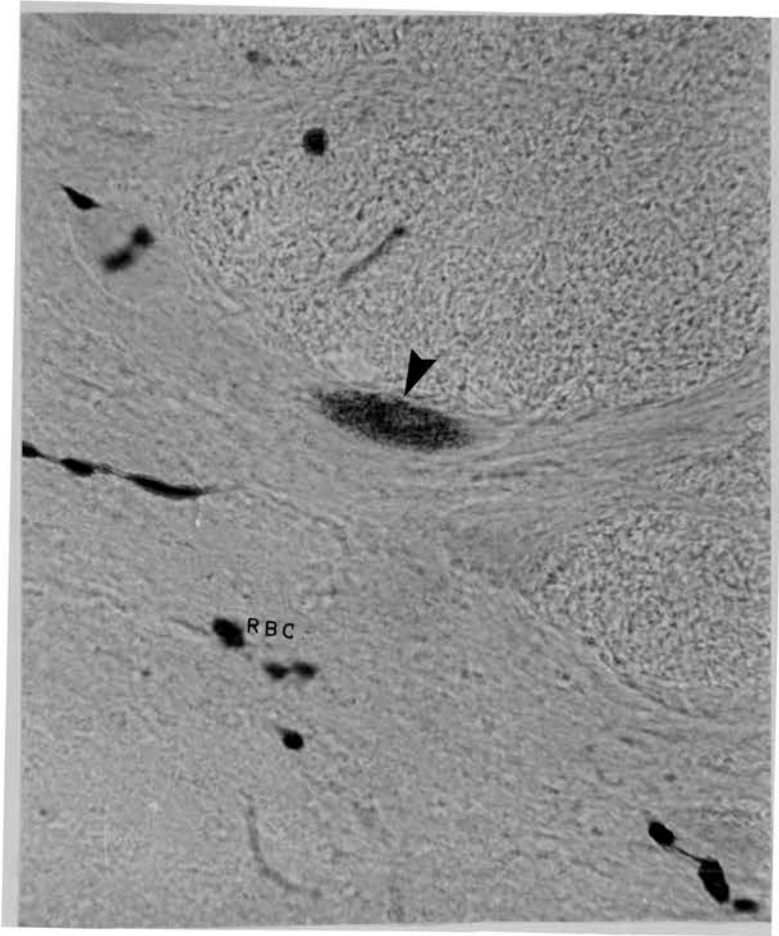


Figure 29. Photomicrograph of frozen section of midbrain of baboon at the level of inferior colliculi (T.S.) showing an HRP labelled fusiform mesencephalic neuron (4) Neutral red stain x300. Red blood corpuscle (RBC).



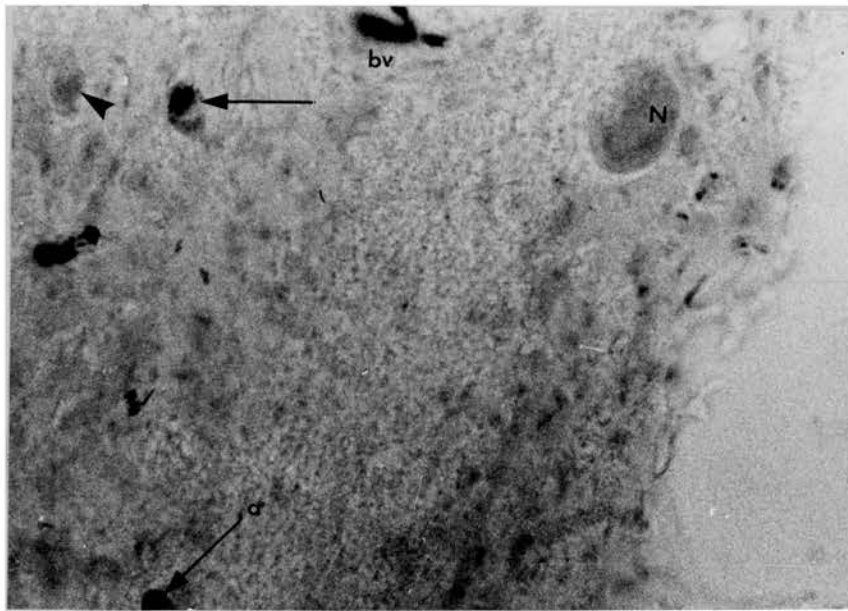
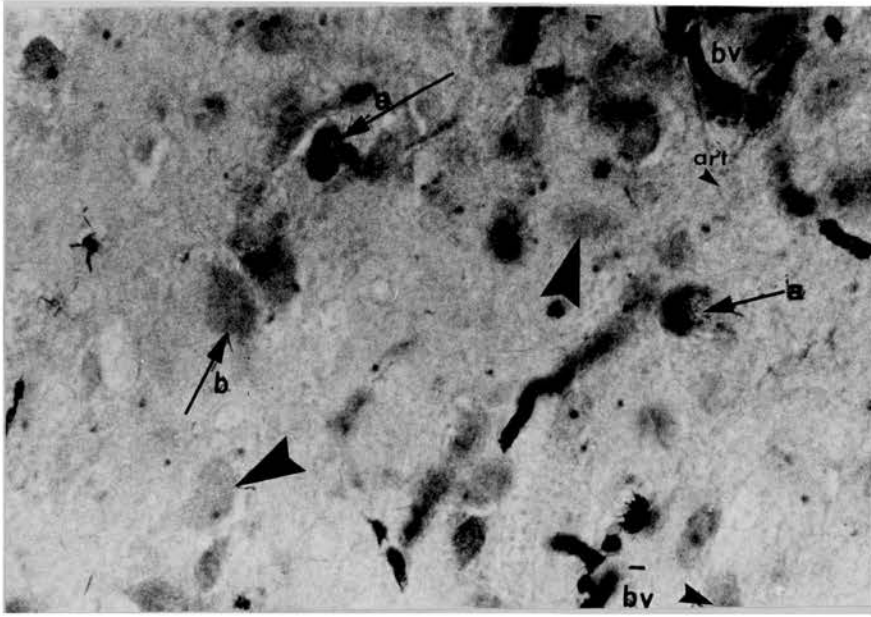
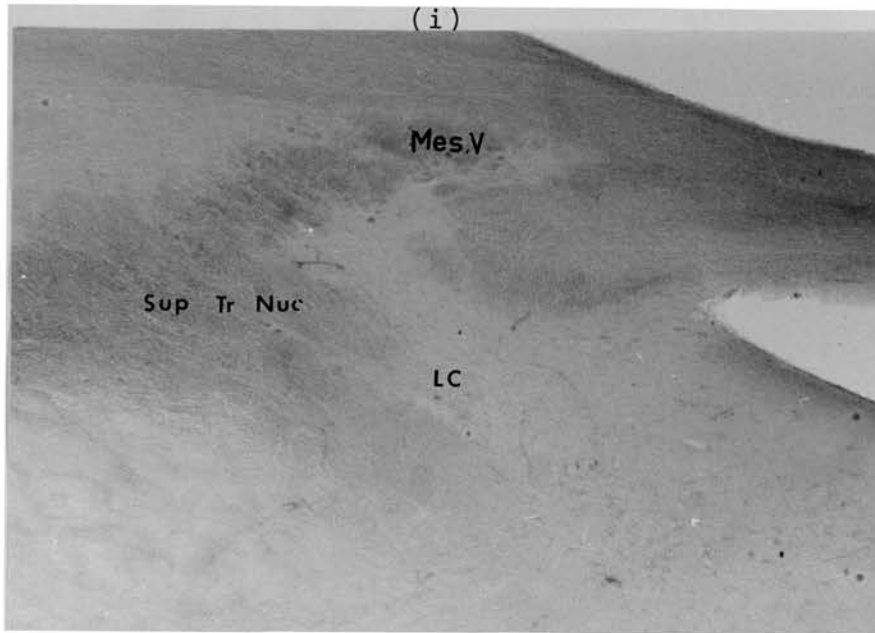
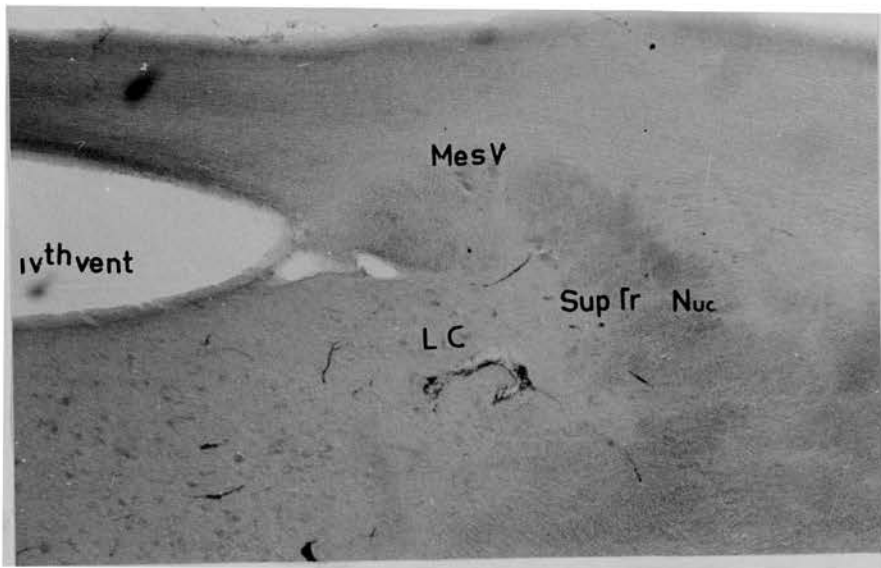


Figure 30. Photomicrographs of frozen sections of pons of baboon (T.S.) at the caudal limit of the mesencephalic nucleus showing smaller HRP labelled mesencephalic neurons (arrows a, densely labelled and b, faintly labelled cells, Unlabelled cells, arrowheads, Large neuron (N) blood vessels (bv), needle-like artifacts. (art). Neutral red stain, x 300.



(ii)

Figure 31. Photomicrographs of (i) right and (ii) left sides of a frozen section of pons in the monkey (T.S.) The faintly labelled cells are seen bilaterally in the region of the supratrigeminal nucleus. (Sup Tr. Nuc). Mesencephalic neurons (Mes V). Locus coeruleus (LC), Fourth ventricle (IV<sup>th</sup> vent). Neutral red stain x 50.



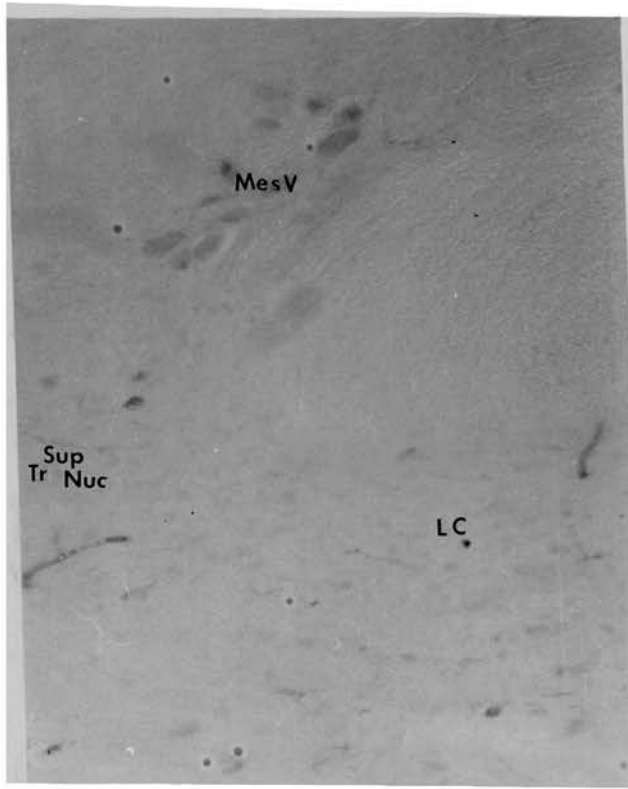


Figure 32. Photomicrograph of frozen section of pons in the monkey (T.S.) showing the mesencephalic neurons (Mes V), the locus coeruleus (LC) and the periphery of supratrigeminal nucleus (Sup. Tr. Nuc.) Neutral red stain. x125.

3D. Quantitative Analysis Of The HRP Labelled Periodontal Mesencephalic Afferent Neurons of the Trigeminal Nerve in the Vervet Monkey and Olive Baboon.

3.35 In the vervet monkeys and olive baboons, a variable number of HRP labelled mesencephalic neurons was found on the ipsilateral side of the nucleus following unilateral HRP injection to the periodontal ligament and gingivae of the maxillary and mandibular incisors, canines and molars (Table 5).

3.36. When HRP was injected into the periodontal ligament and gingivae of the maxillary incisors in the monkey and baboon, the number of labelled cells observed ranged from 13 to 18. Similarly for mandibular incisors the range was 11 to 24. For the maxillary and mandibular canines, the range was 6 to 15 and 12 to 19 respectively. In the maxillary and mandibular molars the range was 6 to 12 and 5 to 18 respectively. It appears, therefore, that the number of labelled neurons is generally higher for the incisor teeth, than the canine teeth and the molars. **The** mandibular teeth also appear to have higher numbers of labelled neurons than the maxillary teeth.

3.37. Under favourable experimental conditions such as adequate concentration of HRP at the receptor site in the periodontal ligament, optimum survival period to allow

accumulation of the retrogradely transported HRP and good fixation of the tissue, the number of HRP labelled neurons observed is high. The parameters of the histochemical reaction and other factors may also influence the number of labelled neurons observed. Thus, in the monkey and baboon, the highest number of labelled neurons observed for the maxillary and mandibular incisors, canine and molars were taken as the 'best counts' shown in table 6. It is apparent that in the monkey and baboon, incisors have a higher number of labelled neurons than the canines and molars and the mandibular teeth have a higher number than the maxillary teeth.

3.38a. The analysis of variance was carried out on the data shown in Table 5 for the ipsilateral mesencephalic neurons using SPSS package; comparing the numbers of labelled neurons associated with different teeth from different jaws in the monkey and the baboon. It was found that the p-value was  $> 0.1$  showing that at 10% level there was no overall significant difference in the number of labelled neurons between the monkey and the baboon and between the various teeth types.

3.38b. The data of the number of labelled mesencephalic neurons for the incisors, canines and molar teeth shown in Table 5 for the monkey and baboon was merged (value of zero was not considered) and mean values and S.D for the various teeth types were calculated. Student t-test was carried out to determine if the difference between the mean number for the various teeth types and between the maxillary teeth and the mandibular teeth was significant. The difference in the mean number of labelled neurons for incisors ( $17 \pm 4$ ,  $n = 7$ ) and molars ( $11 \pm 4.2$ ,  $n = 8$ ) was significant ( $p < 0.02$ ). The differences between incisor and canine mean ( $11.3 \pm 5.1$ ,  $n$

= 6) was also significant, ( $P < 0.05$ ). The difference between canine and molar mean number was not significant ( $p < 0.45$ ). The differences between the means of mandibular and maxillary teeth types were not significant ( $p < 0.2 - 0.35$ ). The coefficient of variation was about 23 % for incisors and 38 - 45 % for canine and molar.

3.39. The distribution of the best counts of labelled neurons along the rostro-caudal extent of the mesencephalic nucleus for the incisors, canines and molars, respectively, in the vervet monkey and olive baboon is shown in Fig. 3.3. More labelled neurons are observed in the caudal part of the nucleus in the region of inferior colliculi and pons. Few labelled neurons are found at the level of the superior colliculi. The pattern of distribution of neurons for the various teeth types is similar in the monkey and baboon and also consistent in the various teeth types.

3.40. The distribution of the total mesencephalic labelled neurons of the best cell counts for the incisors, canines and molars (see Fig.33), together with the mean numbers of unilateral mesencephalic neurons in each 0.5 mm of the brain-stem along the rostro-caudal extent of the mesencephalic nucleus in the monkey and baboon is shown in Fig. 34. The mean value, SD and SE of the unilateral counts of the mesencephalic neurons in the

consecutive 0.5mm thickness of brainstem from rostral to the caudal extent was obtained from observation of serial frozen sections of 50 - 100  $\mu$ m thickness of brainstem of 10 vervet monkeys and 13 olive baboons respectively. The vertical bars in Fig. 34 represent the SE of mean of the unilateral counts.

3.41. The highest density of the labelled periodontal afferents was found caudally in the region of the inferior colliculi and pons and the general pattern of distribution of the labelled neurons for the monkey and baboon appeared to be the same. The best counts for the incisors, canines and molars HRP labelled cells shown in table 6 and the distribution of the labelled and unlabelled neurons in Figs. 33, 34 show that in the vervet monkey, of the total labelled neurons ( $n = 101$ ), 12.9% ( $n = 13$ ) are located in the region of the superior colliculi, 29.7 % ( $n = 30$ ) are located in the region of inferior colliculi and 57.4% ( $n = 58$ ) are located in the region of pons. This shows that in the monkey 87.1 % of the labelled neurons are found in the caudal part of the nucleus. In the baboon, of the total number of labelled neurons ( $n = 83$ ), 12.4% ( $n = 10$ ) are located in the region of superior colliculi, 31.3% ( $n = 26$ ) in the region of inferior colliculi, and 56.6 % ( $n = 47$ ) in the region of

pons. Thus, 87.9% of the labelled neurons are found in the caudal part of the nucleus.

3.42. In the monkey, maxillary teeth accounted for 39.6% (n= 40) of the total labelled neurons and the mandibular teeth accounted for 60.4 % (n = 61). In the baboon, the maxillary teeth accounted for 45.8 % (n = 38) and mandibular teeth 54.2 % (n = 45) of the total labelled neurons.

3.43. Considering the total mesencephalic neurons unilaterally, along the rostro-caudal extent of the mesencephalic nucleus, the mean number of neurons of 10 vervet monkeys was 660 and <sup>of</sup> 13 baboons was 797 (Fig. 34). The HRP labelled periodontal afferent mesencephalic neurons of the maxillary and mandibular incisors, canines and molars resulted in 15.3 % of the total neurons in the monkey and 10.4 % in the baboon. The maxillary incisors, canines and molars accounted for 6.0 % of the total mesencephalic neurons and mandibular teeth 9.3 % of the total cells in the monkey. In the baboon 4.8 % accounted for maxillary teeth and 5.6 % for the mandibular teeth.

3.44. Considering, therefore, the individual teeth types, in the monkey, maxillary and mandibular incisors, accounted for 41.5 % of the total labelled cells and 6.4%

of the total mesencephalic neurons, canines accounted for 28.7% of the labelled cells and 4.4% of the total mesencephalic cells. Molars accounted for 29.7% of the labelled cells and 4.5% of the total cells. In the baboon, incisors accounted for 43.4% of the total labelled cells, and 4.5 % of total cells, canines 32.5 % of total labelled cells and 3.4 % of the total cells and molars 24.1 % labelled cells and 2.5 % of the the total cells.

3.45. The data suggests that the three teeth types are fairly well represented in the mesencephalic nuclues. However, the incisors periodontal afferents have a significantly higher representation than the canines and molars.

Table 5 showing various teeth types periodontal ligament injected with HRP and the number of labelled cells in the trigeminal ganglia and the mesencephalic nucleus in the monkeys and baboons.

Animal	Tooth	(teeth)	types	Trigeminal Ganglion		Ipsilateral Mesencephalic Neurons
				RT	LT	
*Monkey	2	Max. I <sup>1</sup> I <sup>2</sup>	(RT) pdl (intact)	72	-	18
Baboon	880	Max. I <sup>1</sup> I <sup>2</sup>	(RT) pdl (socket)	0	0	0
Baboon	863	Max. I <sup>1</sup> I <sup>2</sup>	(LT) pdl (intact)	12	93	17
Baboon	62	Max. Dec I <sup>1</sup> I <sup>2</sup>	(LT) pdl (intact)	19	103	13
*Monkey	4	Mand I <sub>1</sub> I <sub>2</sub>	(RT) pdl (apical)	70	-	11
"	6	Mand I <sub>1</sub> I <sub>2</sub>	(RT) pdl (apical)	150	19	18
"	10	Mand I <sub>1</sub> I <sub>2</sub>	(RT) pdl (intact)	218	33	24
Baboon	865	Mand I <sub>1</sub> I <sub>2</sub>	(RT) pdl (intact)	150	18	19
Monkey	3	Max Canine	(LT) pdl (intact)	0	85	10
"	8	Max Canine	(LT) pdl (intact)	0	15	6
Baboon	879	Max Canine	(RT) pdl (intact)	108	0	15
*Baboon	864	Max Canine	(LT) pdl (intact)	-	78	6
Monkey	5	Mand Canine	(RT) pdl (apical)	158	0	19
*Baboon	866	Mand Canine	(RT) pdl (intact)	30	0	0
Baboon	59	Mand Dec Canine	(LT) pdl (intact)	6	37	12
*Monkey	4	Max M <sup>1</sup> M <sup>2</sup>	(LT) pdl + apical	-	18	12
Monkey	9	Max M <sup>1</sup> M <sup>2</sup>	(RT) pdl (intact)	37	0	10
Baboon	866	Max M <sup>1</sup> M <sup>2</sup>	(LT) pdl (intact)	-	0	6
Monkey	1	Mand M <sub>1</sub> M <sub>2</sub>	(RT) pdl (socket)	12	0	18
*Monkey	7	Mand M <sub>1</sub> M <sub>2</sub>	(RT) pdl (apical)	10	-	11
Baboon	881	Mand M <sub>1</sub> M <sub>2</sub>	(LT) pdl (socket)	0	0	5
*Baboon	864	Mand M <sub>1</sub> M <sub>2</sub>	(RT) pdl (intact)	15	-	14
Baboon	58	Mand M <sub>1</sub>	(LT) pdl (intact)	0	30	12

\*Monkeys and baboons in which two tooth/teeth types were injected in the right or left sides of the opposing dential arch.



Table 6 showing the "best counts" of the HRP labelled Mes V neurons for the various teeth types in the monkey and baboon (unilateral counts).

<u>M O N K E Y</u>			Total	<u>B A B O O N</u>			Total
Maxillary	Mandibular			Maxillary	Mandibular		
Incisors.	18	24	42	17	19		36
Canine	10	19	29	15	12		27
Molars	<u>12</u>	<u>18</u>	<u>30</u>	<u>6</u>	<u>14</u>		<u>20</u>
Total	<u>40</u>	<u>61</u>	<u>101</u>	<u>38</u>	<u>45</u>		<u>83</u>

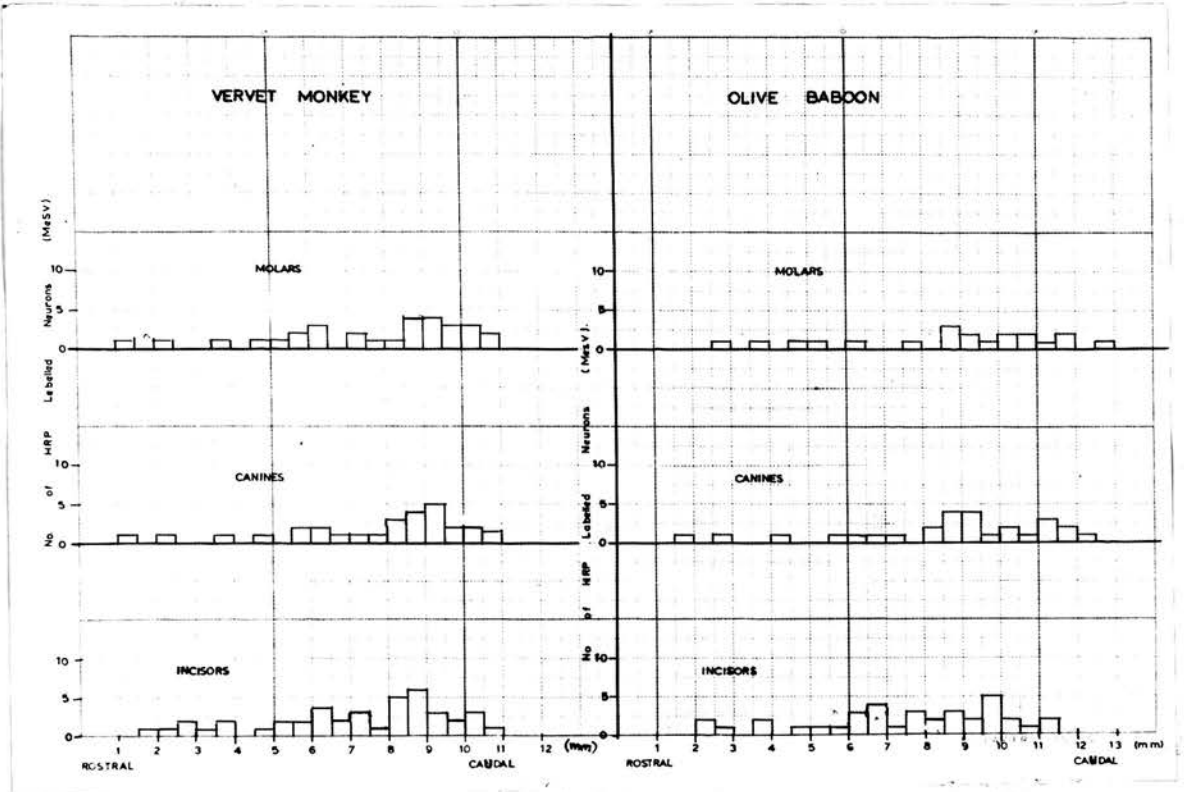


Figure 33. Histograms showing the distribution of number of HRP labelled mesencephalic neurons for the incisors, canines and molar teeth (periodontal ligament/gingivae) along the rostro-caudal extent of the mesencephalic nucleus in the vervet monkey and olive baboon.

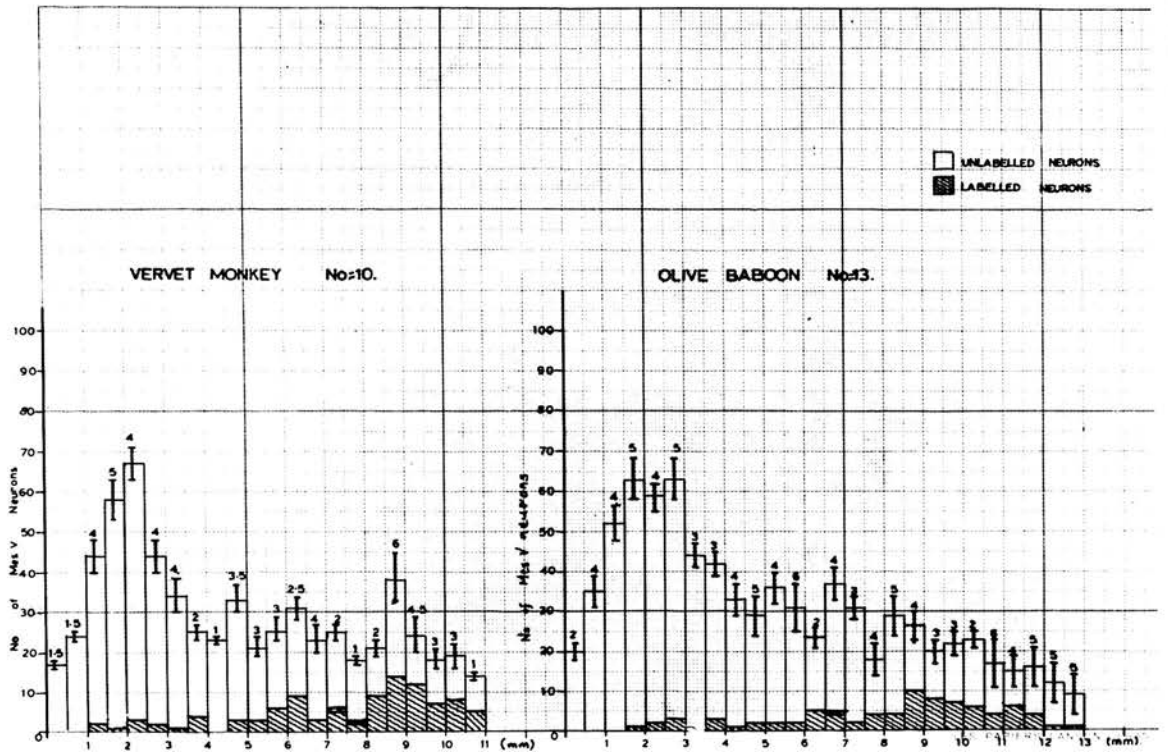


Figure 34. Histogram showing the distribution of the total number of HRP labelled mesencephalic neurons for the three teeth types and the mean number of unlabelled neurons in each 0.5mm of the brainstem along the rostro-caudal extent of the mesencephalic nucleus in the vervet monkey and olive baboon (unilateral counts).

E. Structural Organization Of The Trigeminal Ganglion of  
The Vervet Monkey and Olive Baboon

3.46 The trigeminal ganglion of the vervet monkey and olive baboon consists of an aggregation of neurons forming a crescent shaped band. The central processes of the neurons connect with pons and the peripheral processes leave as three divisions of the trigeminal nerve. The three divisions consist of medially the ophthalmic, laterally the mandibular and in the middle the maxillary division (Fig. 35). The trigeminal ganglion neurons are compactly arranged within the crescent shaped ganglionic band, becoming more dispersed in columns anteriorly where the three divisions of the trigeminal nerve branch off (Figs. 35, 36). Posteriorly, the neurons are compact with the central fibres from the posterior limit of the band converging to form a central core of fibres. The central fibres form the central root of the ganglion (Figs. 35, 36).

3.47 The ganglionic area is composed of the cell bodies, a variable extent of the initial portion of the axon, a ring of satellite cells surrounding the cell body together with intraganglionic fibre bundles and blood vessels (Figs 36, 37). The neurons of the trigeminal ganglion are arranged in long columns separated by fibre bundles along the periphery of the crescent band and are

more compact within the centre of the ganglion (Figs. 36 a, b). The structural organization of the trigeminal ganglion in the vervet monkey and olive <sup>baboon</sup> is found to be very similar. There is variation in size of the neurons within the ganglion. Generally the neurons were medium to large sized spherical pseudounipolar cells (30-70 $\mu$ m). The cell-body has a clearly defined nucleus with a single prominent nucleolus which is usually centrally placed. The cytoplasm contains Nissl granules which are dispersed within the cytoplasm (Fig. 37 a, b). The cell body of the neuron is enclosed within a ring of satellite cells which form a capsule of flattened cells around the perikaryon (Figs. 37 a, b). The initial portion of the axon or the stem process of the unipolar cell process arises from the region of cone-like Nissl free area of the cell body. The stem process has a short intracapsular tortuous course when leaving the cell body. The initial axon or the stem process then divides into centrally and peripherally directed <sup>cell</sup> processes (Figs. 37 a, ). Apart from the neurons and their processes, there are Schwann cells of peripheral nerve fibres and blood vessels in the ganglion (Figs. 37 ab).

3.48. Two types of cells were observed in stained paraffin wax sections. There were generally large lightly stained cells (about 60%) and small to

medium-sized darkly stained cells (about 40%) (Figs.37 a,b). In some of the frozen and paraffin wax sections, lipofuscin pigment granules were observed as yellow clumps at one pole of the cell. At electron microscopic level, there were lipofuscin and membrane bound bodies such as lysosomes observed in the trigeminal ganglion neurons in sections processed for HRP histochemistry.

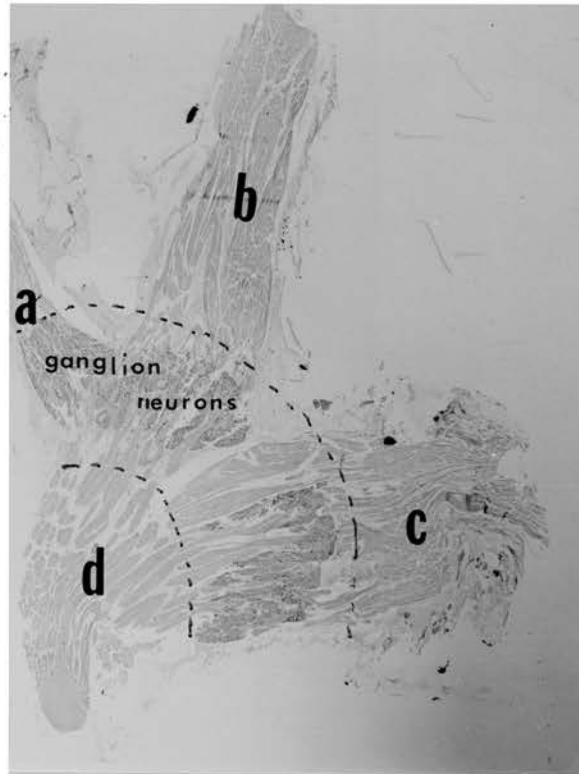
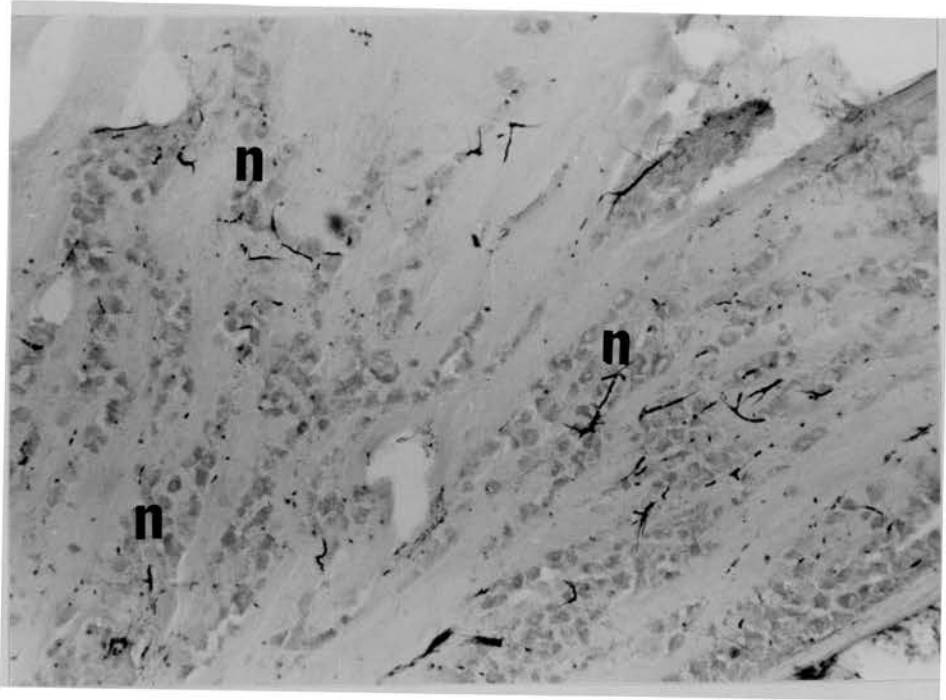
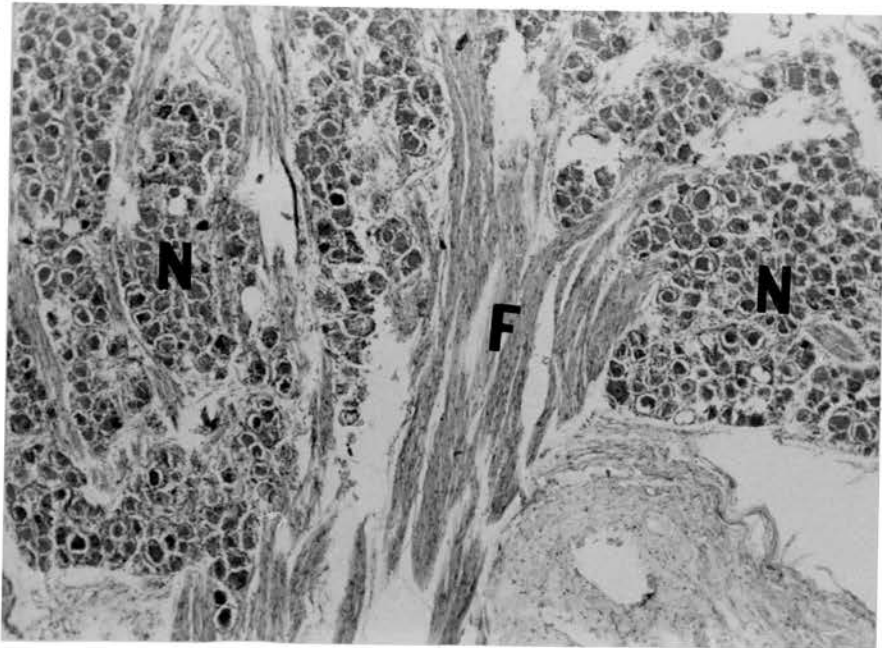


Figure 35. Photomicrograph of paraffin wax section (10 $\mu$ m) of the trigeminal ganglion (T.S.) of monkey showing the middle "ganglionic neuronal region" out-lined, the three divisions of the trigeminal nerve and the central root.  $\times 10$ .  
(a) Ophthalmic division (b) maxillary division  
(c) mandibular division (d) central root.



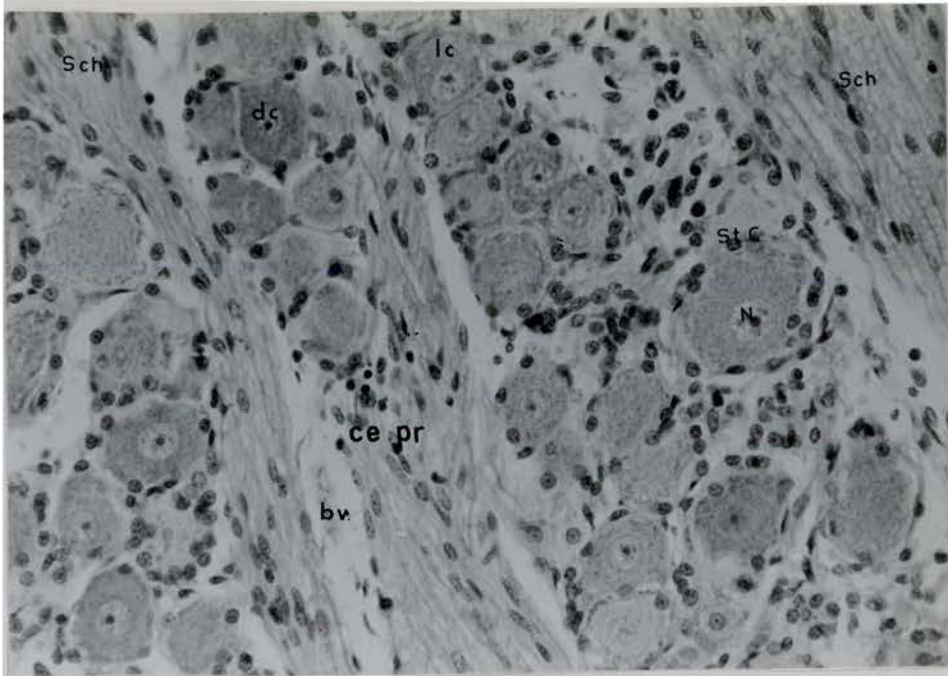
(a)



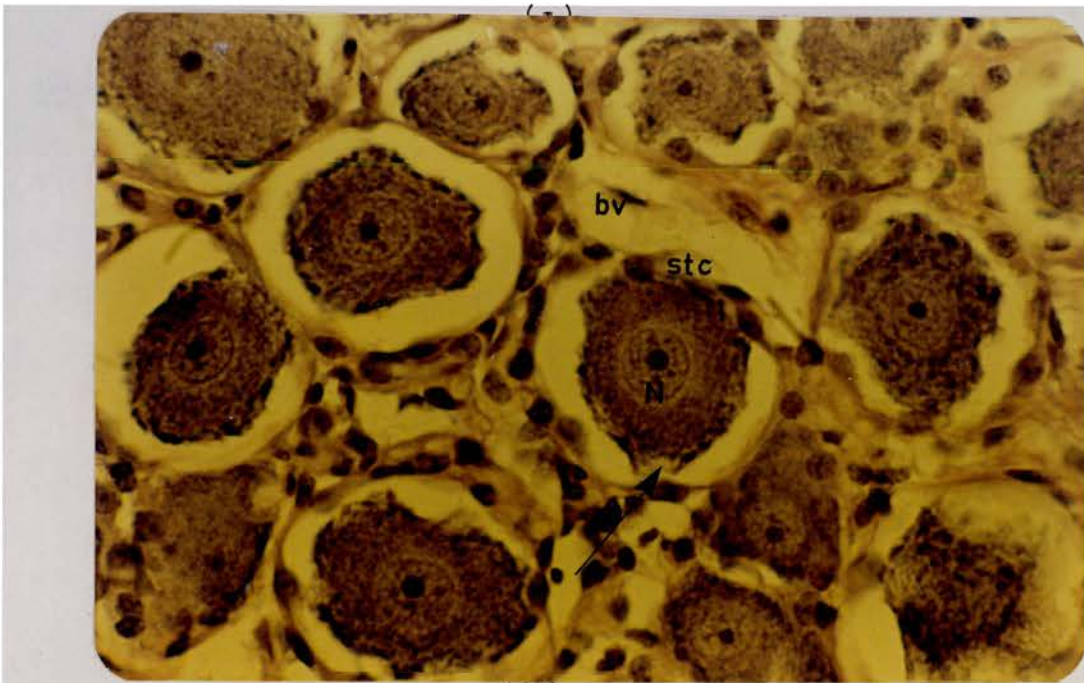
(b)

Figure 36. Photomicrographs (T.S.) of trigeminal ganglia of monkey (a) frozen section (50 $\mu$ m) showing (n) columns of neurons in the periphery of the ganglionic band. Neutral red stain. x50 (b) paraffin-wax section (10 $\mu$ m) showing the (N) compact cells with intraganglionic fibre bundles (F) H/E stain. x50.





(a)



(b)

Figure 37. Photomicrographs of paraffin wax sections of trigeminal ganglia (T.S.) of baboon (a) neurons with a clear nucleus (N) and satellite cell (StC), and cell processes (Ce. Pr.) x 300 (b) Neurons with nucleus and nucleolus and Nissl free stem process (arrow) x 600. Dark cell (d c), light cell (l c), blood vessel (b.v.). Schwann cells (Sch)

F) Quantitative Analysis Of The Trigeminal Ganglion Neuron Of The Vervet Monkey And Olive Baboon.

3.49. The results of the stereological analysis based on stratified serial sections of monkey and baboon trigeminal ganglions are shown in Table 7. The section thickness of the ganglia ranged from 7-10 $\mu$ m and about 10 to 15 sections of each ganglion were analysed. Of the four ganglia of the monkey (a to d) two were from the same monkey (right and left) and two were from different monkeys (wt. 2-4kg). For the baboon, two were from one adult baboon (right and left wt. 16kg) and two from a young baboon, (right and left- wt. 6kg).

3.50. The reference area of the ganglion was the part composed of the neurons referred to as the "ganglionic region". The volume density,  $V_V$  of the neurons in the monkey ranged from 0.30 to 0.35. In the baboon, the  $V_V$  ranged from 0.3 - 0.35 in the adult and 0.40 to 0.45 in the young, with an overall mean of 0.40. There was very little variation in the volume density between the dorso-ventral sections from one ganglion, suggesting that the neurons were generally evenly distributed amongst the intraganglionic fibres and blood vessels. In the analysis of a section, the volume density of neurons was lower in the periphery of the ganglionic band in the anterior region where the three divisions of the nerve

branched off from the ganglion. The mean  $V_V$  shows that about 35-40% of the ganglionic region of the band is composed of neurons with the ring of satellite cells and the rest is made up of the intraganglionic fibre bundles and blood vessels.

3.51. The number of neurons per unit area of the ganglion  $N_A / \text{mm}^2$  shows that there are about 116-133 neurons in one  $\text{mm}^2$  of the ganglionic tissue in the monkey and 124-137 in young baboon and 71-73 in the adult baboon. In the monkey, mean  $N_A$  was  $127 \pm 7$  and baboon  $101 \pm 34$ . There was little variation of  $N_A / \text{mm}^2$  between sections from dorso-ventral extent of the ganglion, the range being 102-158/ $\text{mm}^2$  in the monkey and 60-94/ $\text{mm}^2$  in the adult baboon.

3.52. The range of diameter of the ganglion neuron in the monkey ( $n=100$ ) was 25-65  $\mu\text{m}$  with mean of  $48 \pm 12 \mu\text{m}$ . In the baboon the range of neurons ( $n=100$ ) was 30-80  $\mu\text{m}$  with a mean of  $54 \pm 13 \mu\text{m}$ . Student t-test showed that the difference between the neuron diameter of the monkey and baboon was significant ( $p < 0.001$ ). About 10% shrinkage was noted for the ganglionic tissue. Thus the corrected mean diameter of the monkey neuron would be about 53  $\mu\text{m}$  and of the baboon neuron about 60  $\mu\text{m}$ .

3.53. The numerical density  $N_V$  -- a ranged from 2028-2640 in the monkey with a mean of  $2440 \pm 285/\text{mm}^3$ . The value for  $N_V$  b ranged from 1931 to 2211 with a mean of  $2055 \pm 117$ . The numerical density shows that there are about 2055 - 2430 cell bodies per mm of the ganglion in the monkey (Table 7a).

3.54. In the baboon, the numerical density ( $N_V$  a) was higher in the young baboon 2185-2390 and lower in the adult baboon 1140-1179. The overall mean numerical density was  $1732 \pm 656$ . The value for  $N_V$  b using the corrected diameter of 60um gave an overall mean of  $1518 \pm 571$  neurons per  $\text{mm}^3$  (Table 7b). There are about 2000 neurons / $\text{mm}^3$  in the young baboon ganglion and about 1100 neurons / $\text{mm}^3$  in the adult baboon ganglion.

3.55. The measured volume of the ganglion was corrected for the "non-ganglionic" tissue which comprised about 30% of the ganglion. The corrected volume of the ganglionic tissue multiplied by the  $N_V$  a gave  $N_{T(\text{est})}$  shown in Table 7a for the monkey, and 7b for the baboon. The measured volume of the monkey ganglion ranged from 0.06-0.08 cc. and baboon 0.08 to 0.17 cc. The corrected volume of the "ganglionic" part of ganglion ranged from 0.037 to 0.05 cc. in the monkey and 0.057 for the young baboon and 0.12-0.13cc for the adult baboon. The estimated total number of neurons ranged from 92740 to 105600 in the

monkey and from 124020 to 146040 in the baboon (Table 7a,b).

3.56. Mean number of neurons per section in the 4 ganglia ranged from 1300 to 1500 in the monkey. The range of total cell count as per the total number of sections was 90,000 to 108290 with a mean of  $98072 \pm 7612$  (Table 7a). In the baboon, the mean number of neurons per section ranged from 1460-1700. The range of total cell counts of four ganglia was 137750 to 162650 with a mean of  $153555 \pm 11343$  neurons (Table 7b). The values for the total estimated neurons (NT est) and the total counts (T count) shows that there are about 98073-101178 neurons in the monkey ganglion and 137,250 to 153555 neurons in the baboon ganglion. Student's t-test showed that the difference in the total number of neurons in the monkey and baboon was significant ( $P < 0.01$ ).

Table 7 showing mean values and SD of volume proportion ( $V_V$ ), number per unit area ( $N_A$ ) and the numerical density ( $N_V$ ) of the trigeminal ganglion neurons in (7a) Vervet monkey and (7b) Olive baboon. The estimated total number of neurons  $N_{T(est)}$  and the total cell counts (T count) are also shown.

(a)		<u>Vervet Monkeys</u>						
<u>Monkey</u>		<u>Section thickness</u>	$V_{V_n}$	$N_{A/mm^2}$	$N_{V_a/mm^3}$	$N_{V_b/mm^3}$	$N_{T_{(est)}}$	<u>T count</u>
<u>Ganglion</u>								
a)		7 $\mu m$	0.38	116	2028	1931	101,490	108,290
b)		7 $\mu m$	0.34	133	2622	2211	104,880	96,000
c)	(RT)	10 $\mu m$	0.30	128	2640	2016	105,600	90,000
d)	(LT)	10 $\mu m$	0.36	130	2470	2063	92,740	98,700
Mean			0.35	127	2440	2055	101,178	98,073
SD			0.034	7	285	117	6,903	7,613

(b)		<u>Olive Baboons</u>						
<u>Baboon</u>								
Ganglion		Section thickness	$V_{V_n}$	$N_{A/mm^2}$	$N_{V^a/mm^3}$	$N_{V^b/mm^3}$	$N_{T(est)}$	T count
Young	(RT)	10 $\mu m$	0.45	137	2390	2108	136,980	153,000
Young	(LT)	10 $\mu m$	0.40	124	2185	1908	124,020	127,750
Adult	(RT)	10 $\mu m$	0.35	71	1179	1014	141,960	162,750
Adult	(LT)	10 $\mu m$	0.30	73	1140	1043	146,040	160,720
Mean			0.40	101	1723	1518	137,250	153,555
SD			0.088	34	657	571	10,274	11,343

$$N_{Va} = \frac{(N_A)^{3/2}}{V_{Vn}^{1/2}}$$

$$N_{Vb} = \frac{N_A}{*D + t}$$

\*D = mean diameter of neurons  
monkey 53  $\mu m$  and baboon 60  $\mu m$

t = section thickness



G. General Distribution of the HRP Labelled Trigeminal Ganglion Neurons in the Vervet Monkey and Olive Baboon.

3.57. HRP labelled trigeminal ganglion neurons were observed following HRP injections to the periodontal ligament/gingivae and tooth pulps of various teeth types in the monkey and baboon (Figs.38,39). The HRP labelled neurons were very distinct with fine blue granules filling the cell either completely or partially. The labelled cells were dispersed amongst the unlabelled cells (Figs. 38,39,40). Some of the labelled cells were very densely filled with fine granules visible in the cell process while in others only the cell body appeared to be lightly filled with HRP granules (Figs.38,39). When a densely labelled cell was sectioned through the middle, the region of the nucleus was clearly outlined. When the cell was sectioned through the perikaryon, the outline of the cell was well defined (Figs.38,39,40). The stem process or the initial axon of the unipolar process was visible adjacent to cell body appearing to have a tortuous course before turning sharply and giving rise to the peripeheral process which was often observed to be filled with HRP granules (Figs. 39,40).

3.58. In the lightly labelled cells, the fine HRP granules were fairly distributed within the perikaryon

showing the clear nuclear region in the centre when sectioned through the middle. The fine granules in very lightly labelled cells sectioned through the perikaryon were very faintly visible and required careful observation at higher magnification of X100 - X300 (Fig.38).

3.59. Although the labelled cells generally appeared as single labelled cells, amongst the unlabelled cells, two or three labelled cells were observed adjacent to one another in a region with labelled cells (Figs.41, 43). Of the two or three adjacent labelled cells one may be densely filled while others lightly filled with HRP. The adjacent labelled cells may also be sectioned at different levels, either in the middle or through the perikaryon (Figs.41, 43).

3.60. In freshly TMB processed sections, the HRP granules appeared to be blue or black and tended to become brown with time. The rate and incidence of fading of the HRP granules with time was the same as that observed for the labelled mesencephalic neurons. The stability of HRP granules was longer in sections dehydrated with isopropyl alcohol than with ethyl alcohol. Some of the labelled cells in the sections dehydrated with ethyl alcohol shown in Fig. 41 faded within 4 weeks while the labelled cells in sections



processed with isopropyl alcohol (Fig. 40) remained stable for up to two years.

3.61. The remanent red blood cells within the capillaries in the ganglion appeared as blue black strands and were easily distinguished from labelled cells (Fig. 43). The degree of background blood vessels was much less than in the brainstem since generally ganglia perfused well and were well fixed. Other artifacts such as needle-like processes and brown granular sediment was less frequently observed in the trigeminal ganglion sections.

3.62. In the monkey and baboon, following HRP injections to the periodontal ligament and gingivae and tooth pulps of mandibular and maxillary incisors, canines and molars, labelled neurons were observed distributed in the respective mandibular and maxillary "compartments" of the trigeminal ganglion. For the mandibular teeth, the labelled neurons were found mainly in the lateral region of the ganglion, along its dorso-ventral and antero-posterior extent but concentrated more in the postero-lateral aspect of the ganglion (Figs. 41.42). The labelled neurons of the mandibular incisors, canines and molars were generally distributed within the mandibular region of the ganglion (Fig.42).

3.63. For the maxillary teeth, the labelled neurons were generally distributed in the middle of the ganglion along the dorso-ventral and antero-posterior extent (Figs. 43, 44). The labelled neurons of maxillary incisors, canines and molars were generally distributed within the maxillary region (Fig. 44).

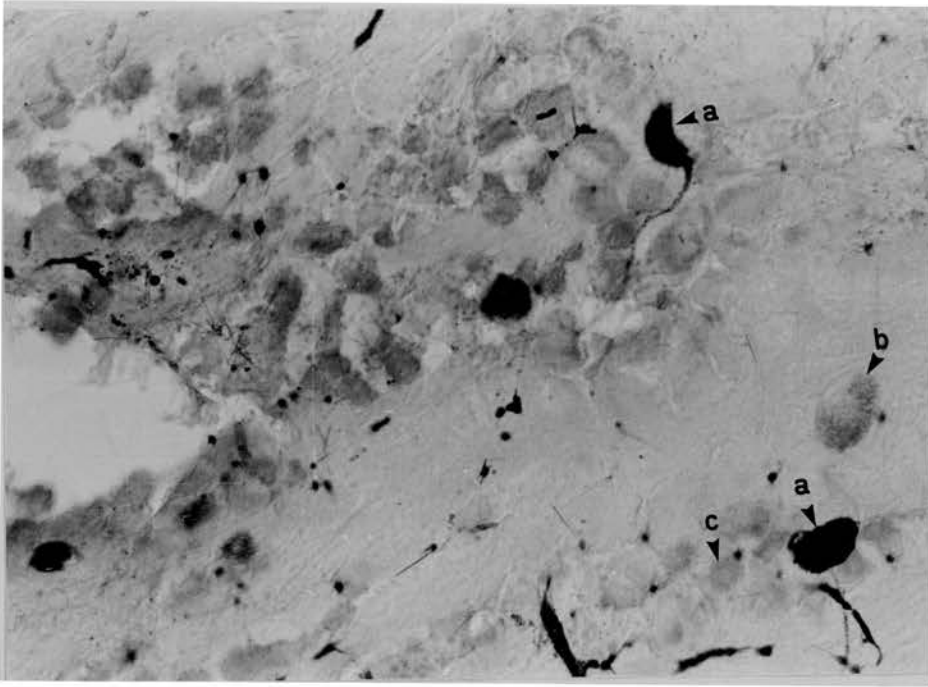
3.64. There was no strict somatotopy or localization for the individual teeth types within the respective maxillary and mandibular compartments of the ganglion since the labelled neurons were distributed throughout the respective regions (Figs. 41-44).

3.65. The density of labelling was higher in the ventral and central part of the ganglion compared to the dorsal part for incisors and canines in some of the monkeys and baboons. In other animals, there was even distribution of labelled cells in the dorso-ventral extent for the incisors and canines. For the molar teeth, the distribution of labelled neurons was generally sparse, but more evident in the dorsal and central part in some monkeys and baboons and evenly distributed in other animals. There was no clear pattern that the afferent neurons of anterior teeth were located generally in the ventral and central part of the ganglion and those of the posterior teeth in the dorsal and central part of the ganglion.

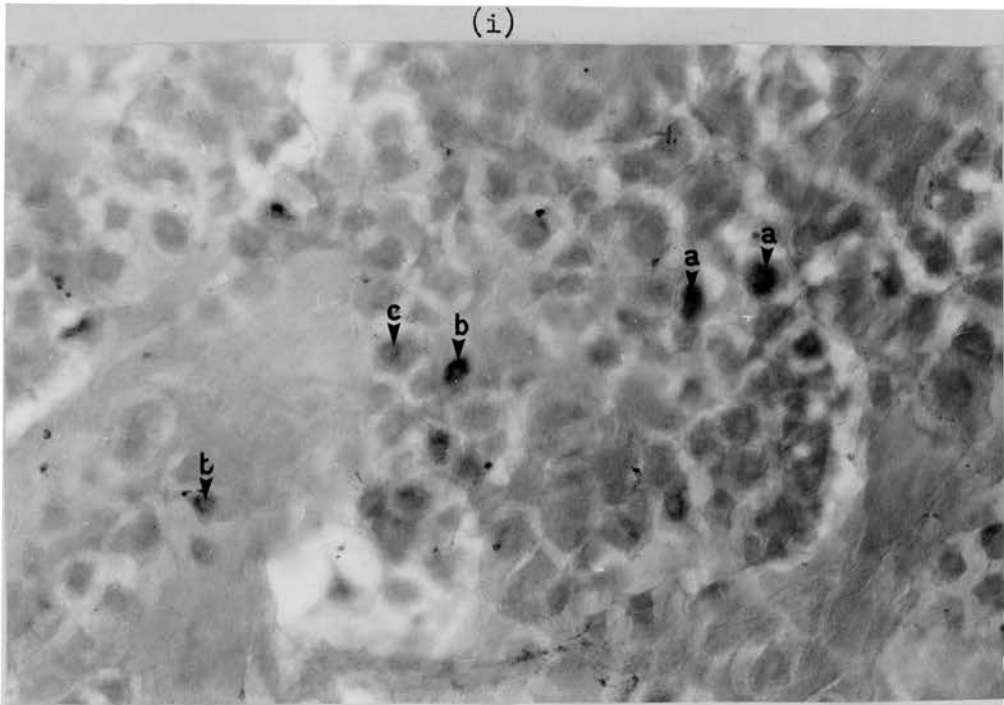
3.66. Although the HRP labelled neurons were mainly within the maxillary compartment for maxillary teeth and the mandibular compartment for the mandibular teeth, there was some degree of overlap in the region of the branching of the maxillary division (Figs. 41-44). The distribution of the labelled periodontal afferent neurons of mandibular teeth diminished from the postero-lateral side towards the maxillary region and similarly, the periodontal maxillary teeth afferent labelled neurons tended to diminish towards the mandibular and ophthalmic regions. One notable difference was when the maxillary incisors tooth pulps were injected with HRP. There was very dense concentration of labelled neurons observed in the middle compartment of the ganglion along the antero-posterior extent. In addition, there were some labelled neurons observed within the ophthalmic region of the ganglion (Fig. 45)

3.67. The labelled neurons were observed mainly in the ipsilateral trigeminal ganglion following unilateral HRP application to periodontal ligament, gingivae and pulp of the various teeth types. Many densely HRP labelled neurons were evident in the ipsilateral trigeminal ganglion and few lightly labelled neurons in the contralateral ganglion following HRP application to maxillary and mandibular incisors. Contralateral

labelling was observed only in one animal where a mandibular canine was injected with HRP. For molar teeth, maxillary and mandibular, there was no labelling observed in the contralateral ganglion. Dense labelling of both trigeminal ganglia neurons was observed in the respective compartments when different teeth types in the opposing arch were injected with HRP in the same animal.



(i)



(ii)

Figure 38. Photomicrographs of frozen sections of trigeminal ganglia (i) of monkey (HRP-pd1/gingivae) x300 and (ii) baboon (HRP-dental pulp) x125, showing HRP labelled trigeminal ganglion neurons. (a) densely labelled neuron sectioned through the perikaryon with HRP granules in cell process. (b) less densely labelled neuron sectioned through the perikaryon. (c) faintly labelled neuron. Neutral red stain.

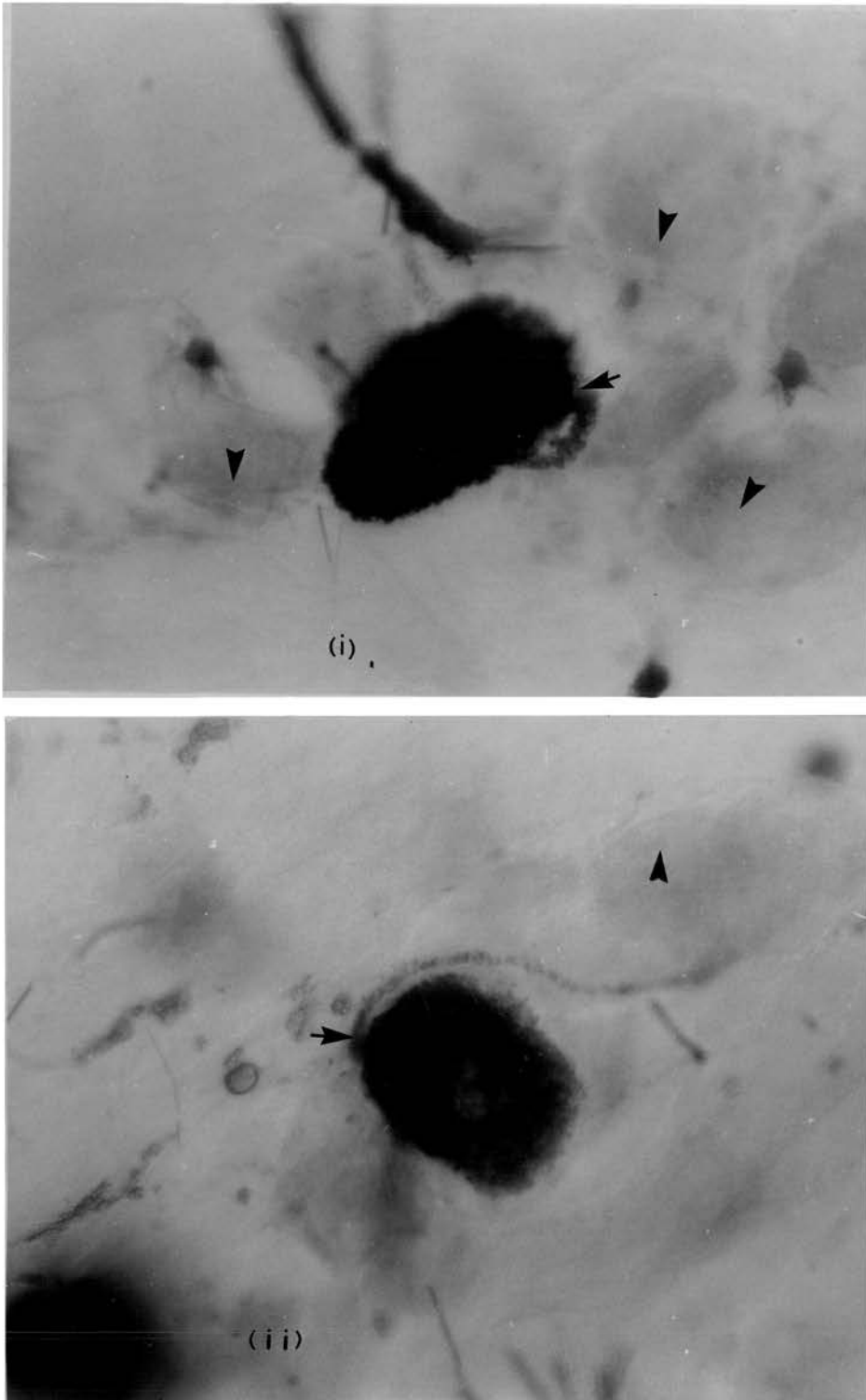
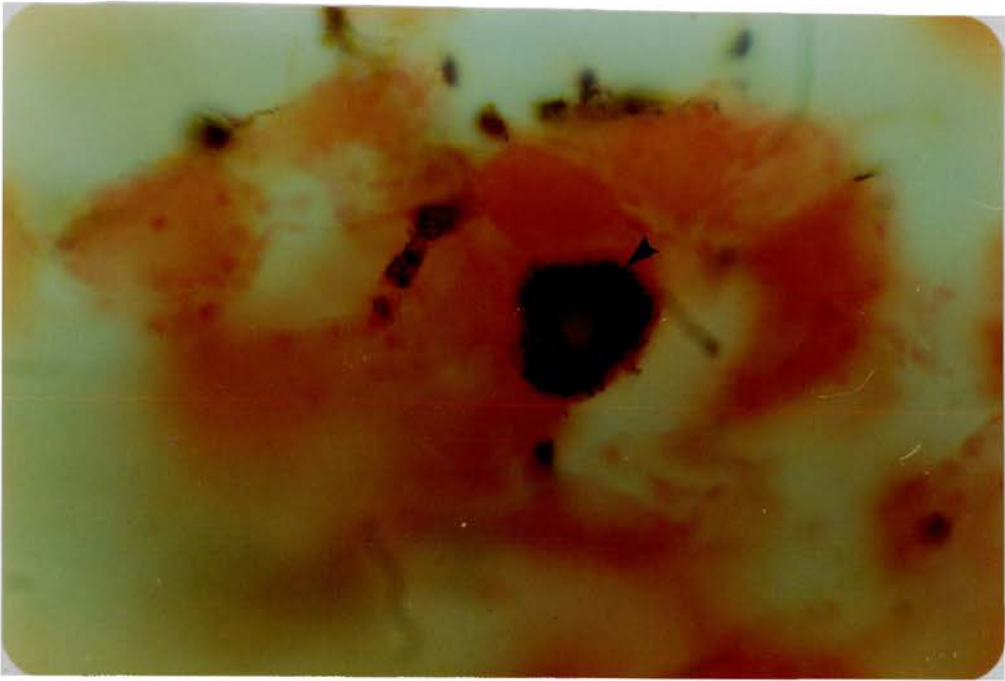
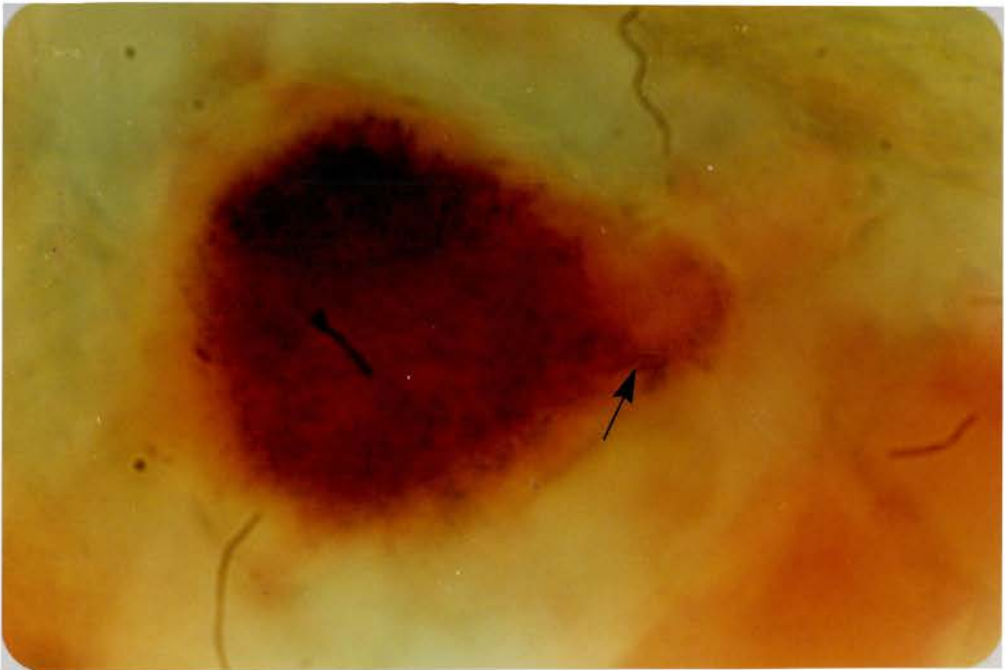


Figure 39. Photomicrographs of frozen sections of trigeminal ganglion showing HRP labelled neurons with the "stem process" (arrow) turning sharply from the cell body. Unlabelled neurons (▲) lie adjacent to the labelled cells (i) and (ii) x600. Neutral red stain.



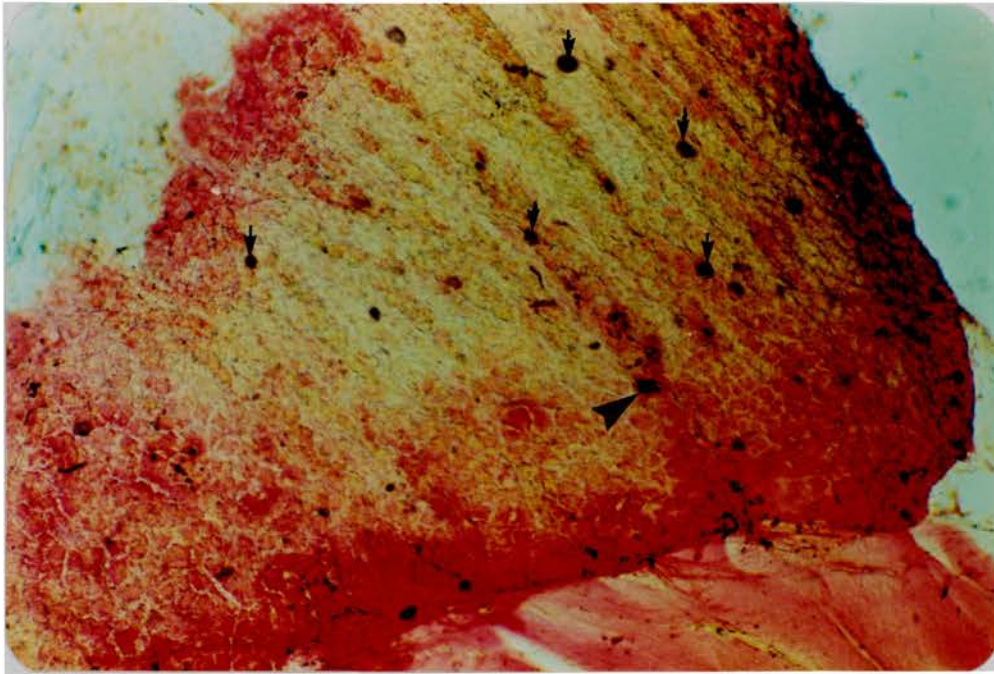
(a)



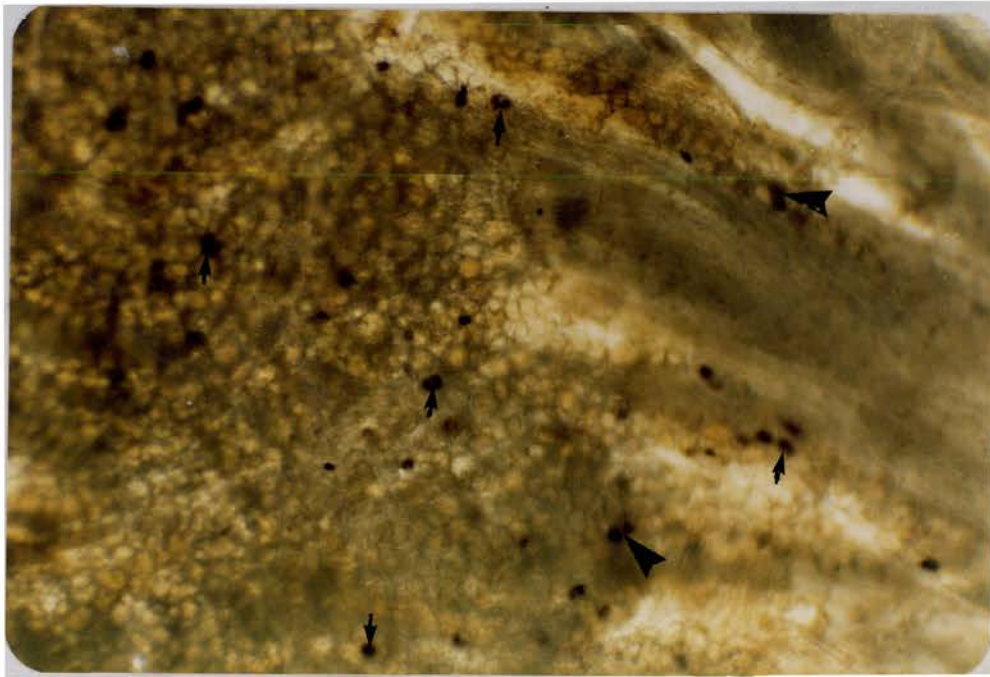
(b)

Figure 40. Photomicrographs of frozen sections of trigeminal ganglion of baboon showing (a) densely labelled neuron sectioned through the centre (▲) x300 and (b) neuron with HRP granules in the cell body and stem process (arrow) x1250. Neutral red stain.





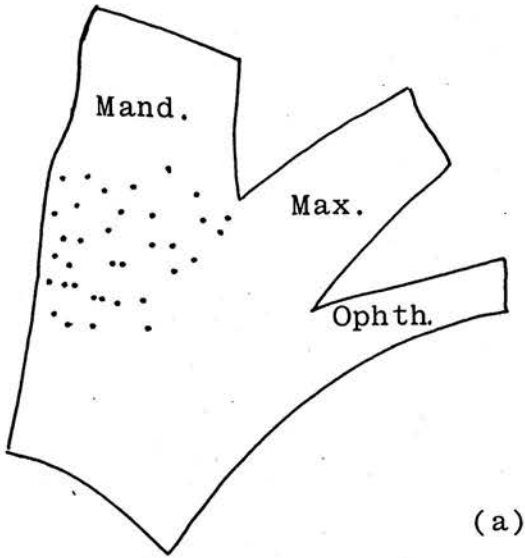
(a)



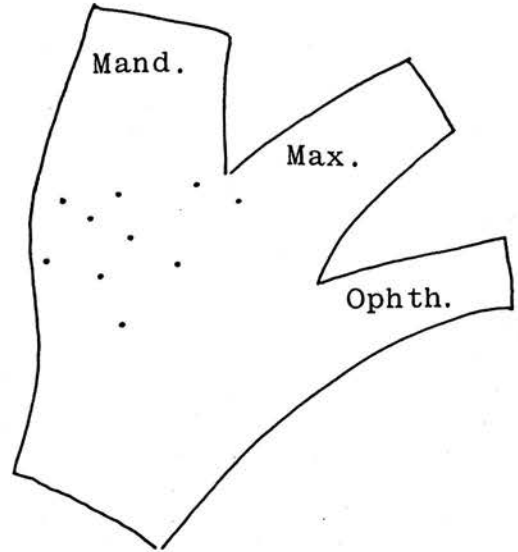
(b)

Figure 41. Photomicrographs of frozen sections of monkey trigeminal ganglia showing the distribution of HRP labelled cells (arrows) in the mandibular region from mandibular teeth periodontal afferents. Arrowhead shows two adjacent labelled neurons. (a) neutral red stain. (b) unstained. x50.

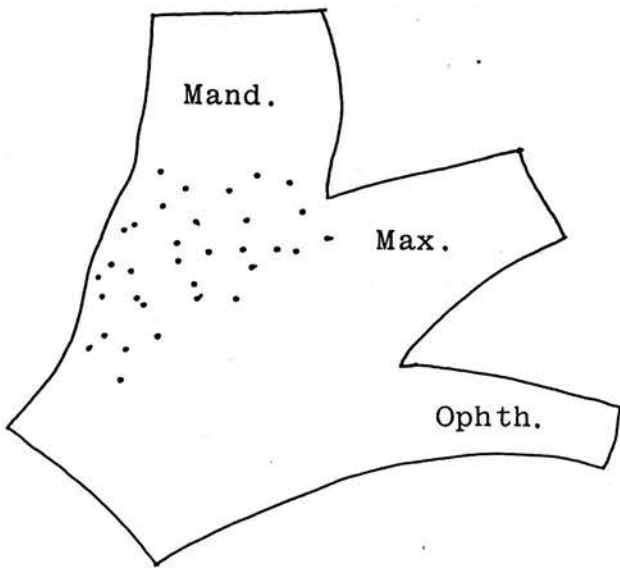




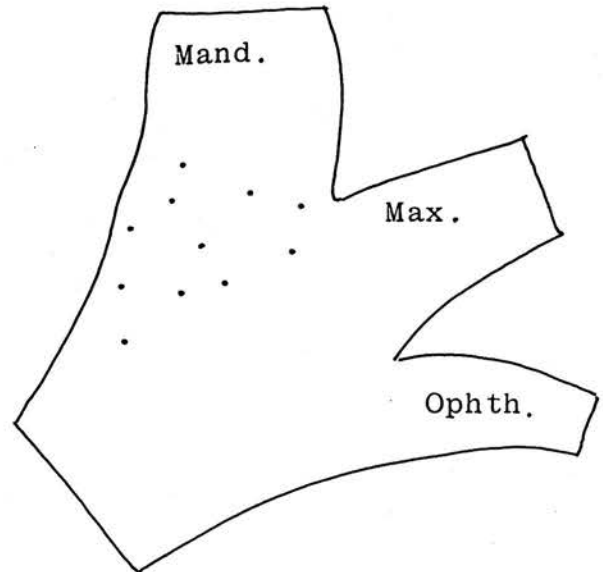
Mand.  $I_1$   $I_2$  and C (pdl)  
Monkey 5, 6, 10



Mand  $M_1$   $M_2$  (pdl) Monkey 7  
Mand.  $M_1$  (pulp) Monkey 2

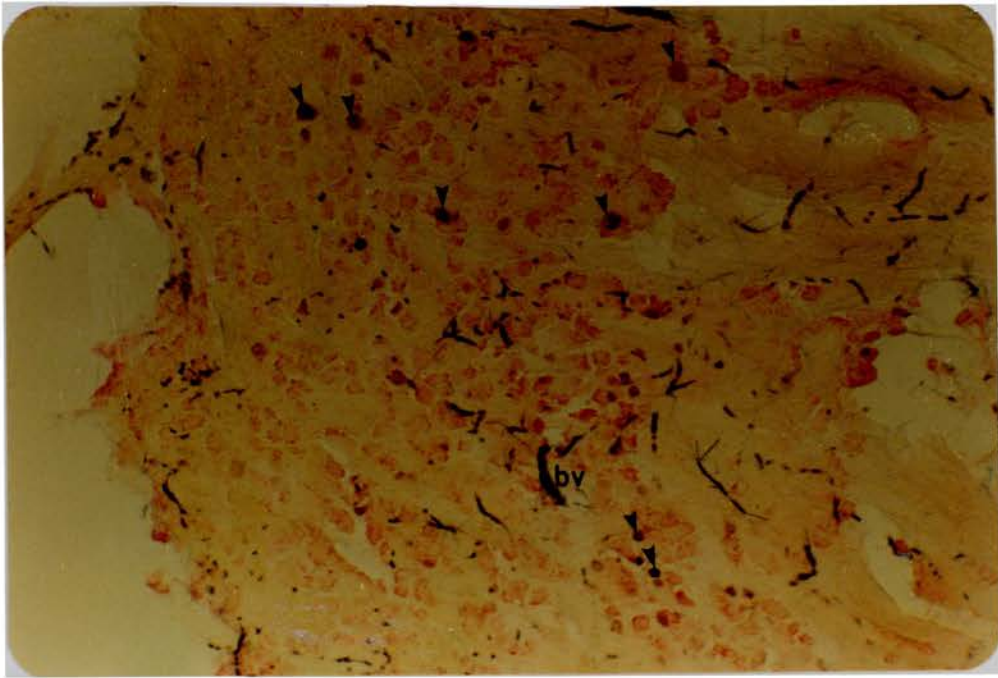


Mand.  $I_1$   $I_2$  C (pdl)  
Baboon 865, 866 and 59  
Mand.  $I_1$   $I_2$  (pulp) Baboon 60

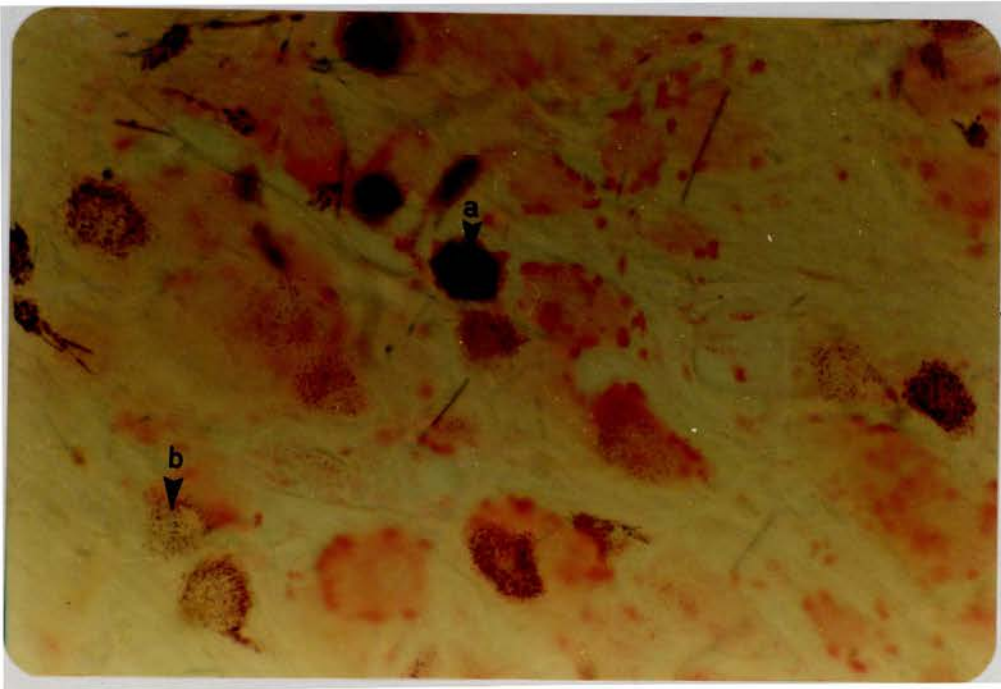


Mand.  $M_1$   $M_2$  (pdl) Baboon 864  
Mand.  $M_1$  (pulp) Baboon 33.

Figure 42 Schematic drawings of the trigeminal ganglion showing the distribution of the HRP labelled trigeminal ganglion afferent neurons of the mandibular teeth in (a) vervet monkey and (b) olive baboons.



(a)



(b)

Figure 43. Photomicrographs of frozen section of trigeminal ganglion of monkey showing the distribution of HRP labelled cells in the maxillary region from maxillary teeth periodontal afferents. (a) densely labelled cell. (b) lightly labelled cell. Neutral red stain. (i) x50 (ii) x300. Blood vessel (bv).

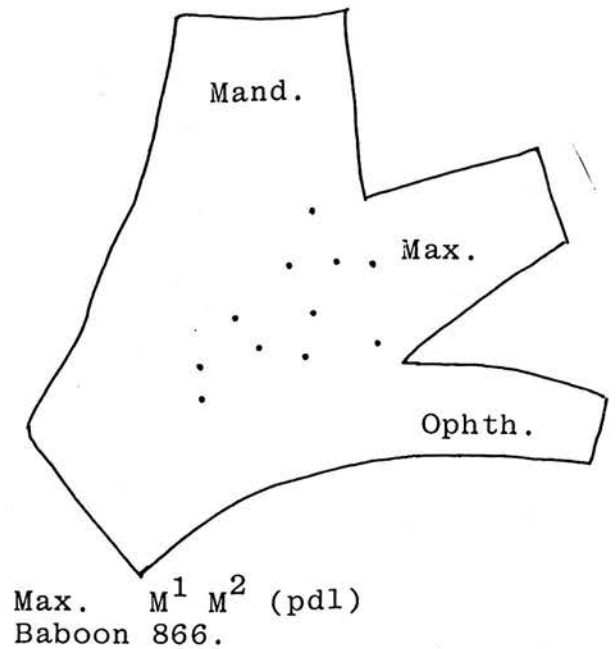
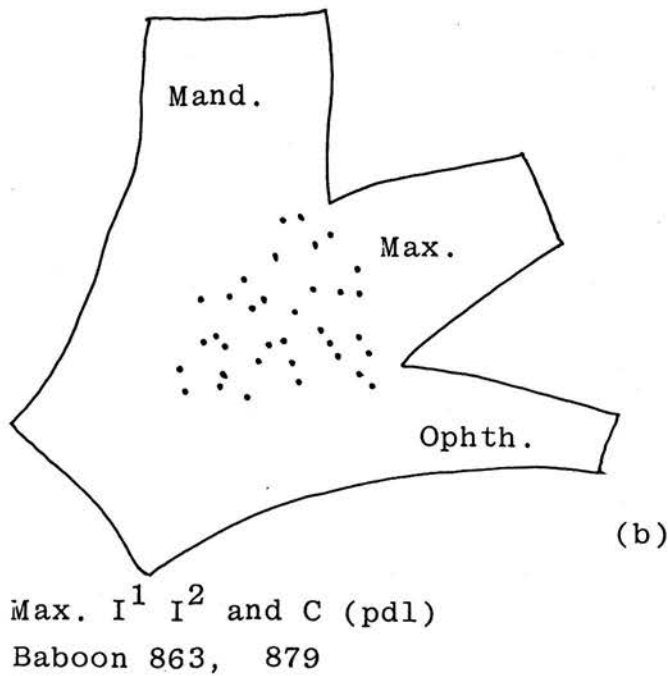
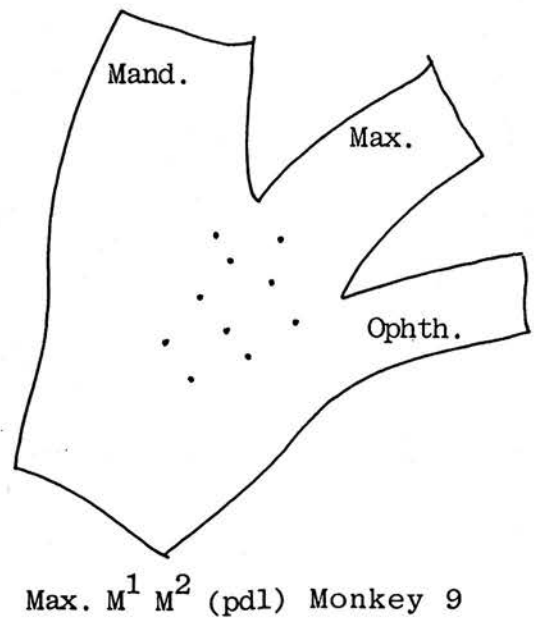
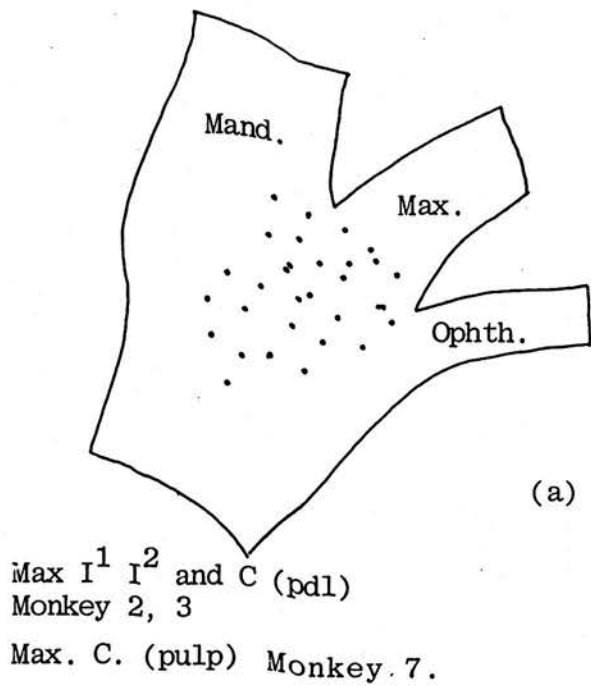


Figure 44 Schematic drawings of the trigeminal ganglion showing the distribution of the HRP labelled trigeminal ganglion afferent neurons of the maxillary teeth in (a) vervet monkey (b) olive baboon.

Max. I<sup>1</sup> I<sup>2</sup> (pulp) Baboon 30

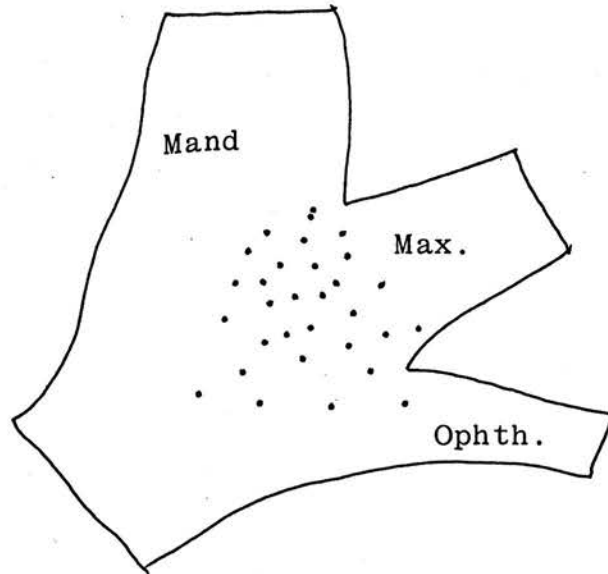


Figure 45 Schematic drawing of the trigeminal ganglion showing the distribution of the HRP labelled trigeminal ganglion neurons of the afferents of maxillary incisor pulp.

H Quantitative Analysis Of The HRP Labelled Neurons In The Trigeminal Ganglion In The Vervet Monkey And Olive Baboon.

Periodontal Ligament/Gingivae.

3.68 In the vervet monkey and olive baboon, a variable number of HRP labelled neurons was found mainly in ipsilateral trigeminal ganglion following HRP injection into the periodontal ligament and gingivae of maxillary and mandibular incisors, canines and molars. The number of HRP labelled trigeminal ganglion neurons observed in the right and left trigeminal ganglia in the monkeys and baboons following HRP injection into the various teeth types are shown in Table 5.

3.69 There is a variation in the number of the labelled neurons observed in the ipsilateral ganglion in different animals where similar teeth types were injected. The range for the maxillary incisor was 72 - 103 in the ipsilateral ganglion with 12 - 19 labelled neurons in the contralateral ganglion. In mandibular incisors the range was 70 - 218 in the ipsilateral ganglion and 18 - 33 in the contralateral ganglion. The range for the maxillary canine range was 15 - 108 in the ipsilateral ganglion. In the mandibular canine the range was 37 - 158 in the ipsilateral ganglion and in one case, 6 neurons were found in the contralateral ganglion.. For the maxillary

molars, the range was 12 - 37 and the mandibular molars, range was 10 - 30 in the ipsilateral ganglion only.

3.70 Although there was variability in the number of labelled neurons when similar teeth types were injected, it appears that the number of labelled neurons is generally higher for the incisors and canine teeth compared to the molars in the monkey and baboon. Mandibular incisors and canines appear to have a higher number of labelled neurons than the maxillary teeth in the monkey. The highest number of labelled ganglion neurons observed for the maxillary and mandibular incisors, canines and molars in the monkey and baboon were taken as "best counts" shown in Table 8 similar to the highest number of labelled mesencephalic neurons.

3.71 Many labelled neurons in a field were observed in a section of the ganglion following HRP application to the periodontal ligament and gingivae of incisors and canine than that of the molars (Fig 46). The distribution of the number of HRP labelled neurons along the dorso - ventral extent of the ganglion for incisors, canine and molars "best counts" in the monkey and baboon are shown in Fig 47. The number of labelled neurons increases towards the centre of the ganglion in both the monkey and baboon. For the incisors and canines, the number of labelled neurons are a little less on the dorsal side than on the

ventral side. However, the difference in the dorso - ventral number was not marked in the distribution of the neurons in all the ganglia observed following HRP to the incisors and canines. The distribution of the fewer number of labelled neurons for the molars showed a somewhat higher number of labelled neurons in the dorsal than the ventral region, although this pattern was not consistent for all the ganglia where molar teeth were injected with HRP.

3.72 The estimation of the mean total number of trigeminal ganglion neurons from analysis of the paraffin wax sections was 101178 in the monkey and 137250 in the baboon. The total number of labelled neurons for the best counts was 582 in the monkey and 440 in the baboon (Table 8). This implies that in the monkey, 0.58% of the total neurons are labelled while in the baboon, 0.32% of the total neurons are labelled.

3.73 In the monkey, incisors represent 0.29%, canines 0.24% and molars 0.05% of the total ganglion neurons, Maxillary teeth account for 0.2% and mandibular for 0.38% of the total ganglion neurons. Of the total labelled neurons, incisors account for about 50%, canines 42% and molars 8%. Of the total labelled neurons, maxillary teeth account for 33.3% and mandibular teeth 66.7%. Mandibular incisors alone account for 37.5%.



3.74 In the baboon, incisors represent 0.18%, canines 0.11% and molars 0.03% of the total ganglion neurons. Maxillary and mandibular teeth account for 0.16% respectively of the total neurons. of the labelled neurons, incisors account for 57.5%, canines 33% and molars 9.5% Maxillary teeth account for 51% and mandibular teeth 49%. Mandibular incisors account for 34% of the labelled neurons.

3.75a Analysis of variance was carried out on the data shown in Table 5 for the trigeminal ganglion neurons using SPSS package, comparing the numbers of ipsilateral labelled neurons associated with different teeth in the monkey and baboon. It was found that the p-value was  $> 0.1$  showing that at 10% level there was no overall significant difference between the monkey and the baboon and between the various teeth types. The F-ratio (3.47) for the teeth types grouped in three, incisors, canines, and molars was very close to the Table value (3.81).

3.75b The data on the number of labelled neurons obtained in the monkey and baboon for incisors, canines and molars was pooled to determine if the differences in the mean numerical counts for the various teeth types were significant. The difference between the incisors ( $n = 7$ ) mean  $122.3 \pm 53.5$  and molars ( $n = 7$ ) mean  $19 \pm 10$  was highly significant ( $p > 0.001$ ). The difference between incisor and canine ( $n = 7$ ) mean  $73 \pm 50$  was not significant ( $p < 0.2$ ). The difference between canine and molar was significant ( $p < 0.01$ ). The differences between the mandibular and maxillary incisors, were significant ( $p < 0.005$ ) while between the two canines and molars were not significant ( $p < 0.03 - 0.35$ ).

3.76 The volume densities of the HRP labelled neurons and the total neurons obtained from the stereological analysis of 3-5 serial frozen section of the trigeminal ganglion of 6 monkeys are shown in Table 9. The sections analysed were of monkeys in which

incisors, canines and molars periodontal ligament and gingivae were injected with HRP as shown in table 9. The volume percent of the labelled neurons shows that for the mandibular incisors, 1.3 - 1.7% cells were labelled, the maxillary incisors 1.1% were labelled, and in the mandibular canine 1.7% and in the maxillary molars 0.15-0.2% were labelled. These values are higher than those obtained for incisors (0.29%) canines (0.24%) and molars (0.05%) considering the percentage of number of labelled neurons out of the total number of ganglion neurons of the monkey.

#### Dental Pulp:

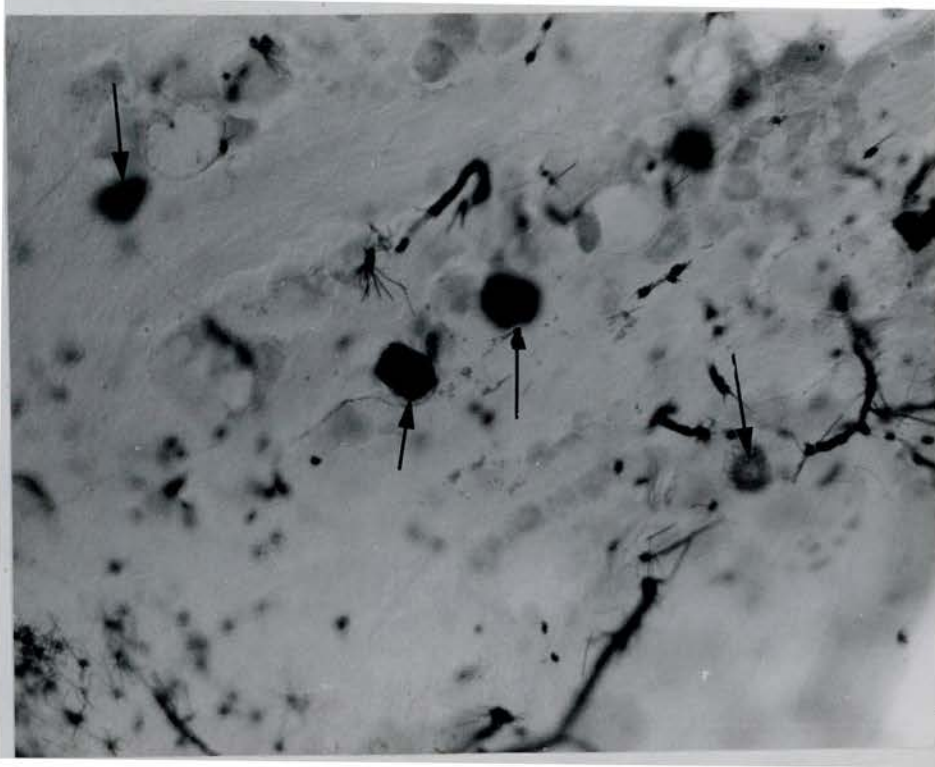
3.77 The number of HRP labelled neurons observed in the trigeminal ganglion of the monkey and baboon following HRP application to the incisors, canine and molar teeth pulps are shown in table 10. The number of labelled neurons observed is higher in the incisor than the canine or molar in a small sample of teeth types studied. The low number for the maxillary canine was noted in the left ganglia of a monkey which was injected with HRP in the left canine through the buccal cavity preparation. The molars showed a smaller number of labelled neurons while incisor pulps tended to have a higher representation. In the baboon for maxillary incisor teeth pulps, 0.66% (n=900) trigeminal ganglion neurons were labelled compared to 0.073% (n=100) for the mandibular molar

taking the total neuronal count of the ganglion to be 137250.

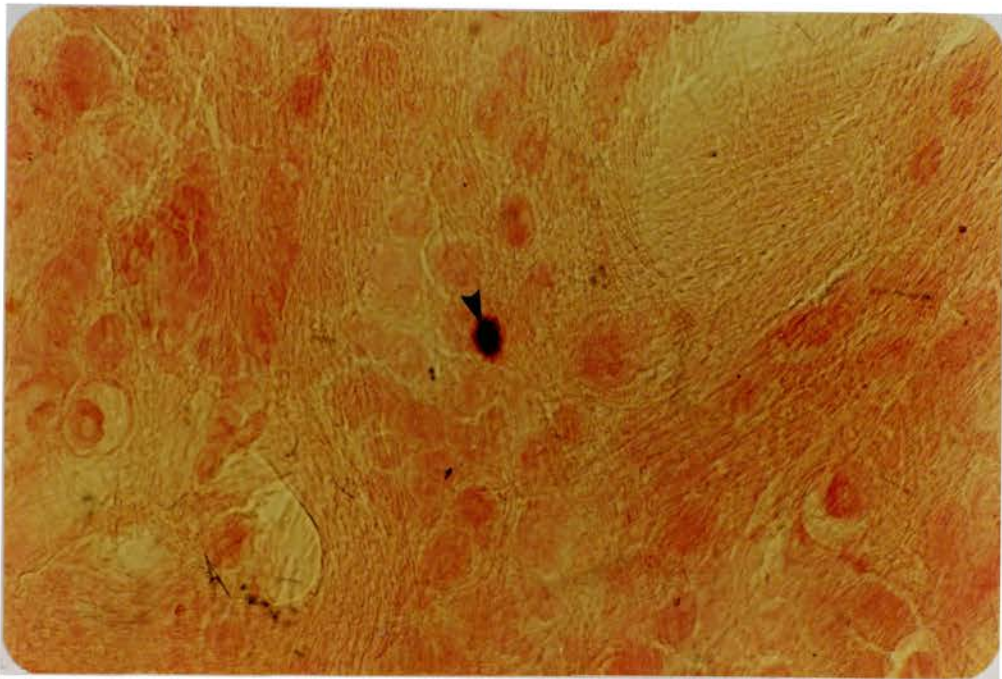
3.78 Many more labelled neurons were observed in the ipsilateral trigeminal ganglion for the maxillary incisor pulp (900) and some in the contra-lateral ganglion (100). The labelled neurons were observed only in the ipsilateral ganglion for the mandibular incisors, maxillary canine and mandibular molars.

Table 8 showing the "best counts" of the labelled trigeminal ganglion neurons for the maxillary and mandibular incisors, canines, and molars in the vervet monkey and olive baboon.

Monkey				Baboon		
	Max	Mand	Total	Max	Mand	Total
Incisors	72	218	290	103	150	253
Canine	85	158	243	108	37	145
Molars	37	12	49	12	30	42
Total	194	388	582	223	217	440



(a)



(b)

Figure 46. Photomicrographs of frozen sections of trigeminal ganglia of monkey showing (a) many (↔) HRP labelled neurons of incisors and (b) single (▼) HRP labelled neuron of molar periodontal afferents. Neutral red stain x125.

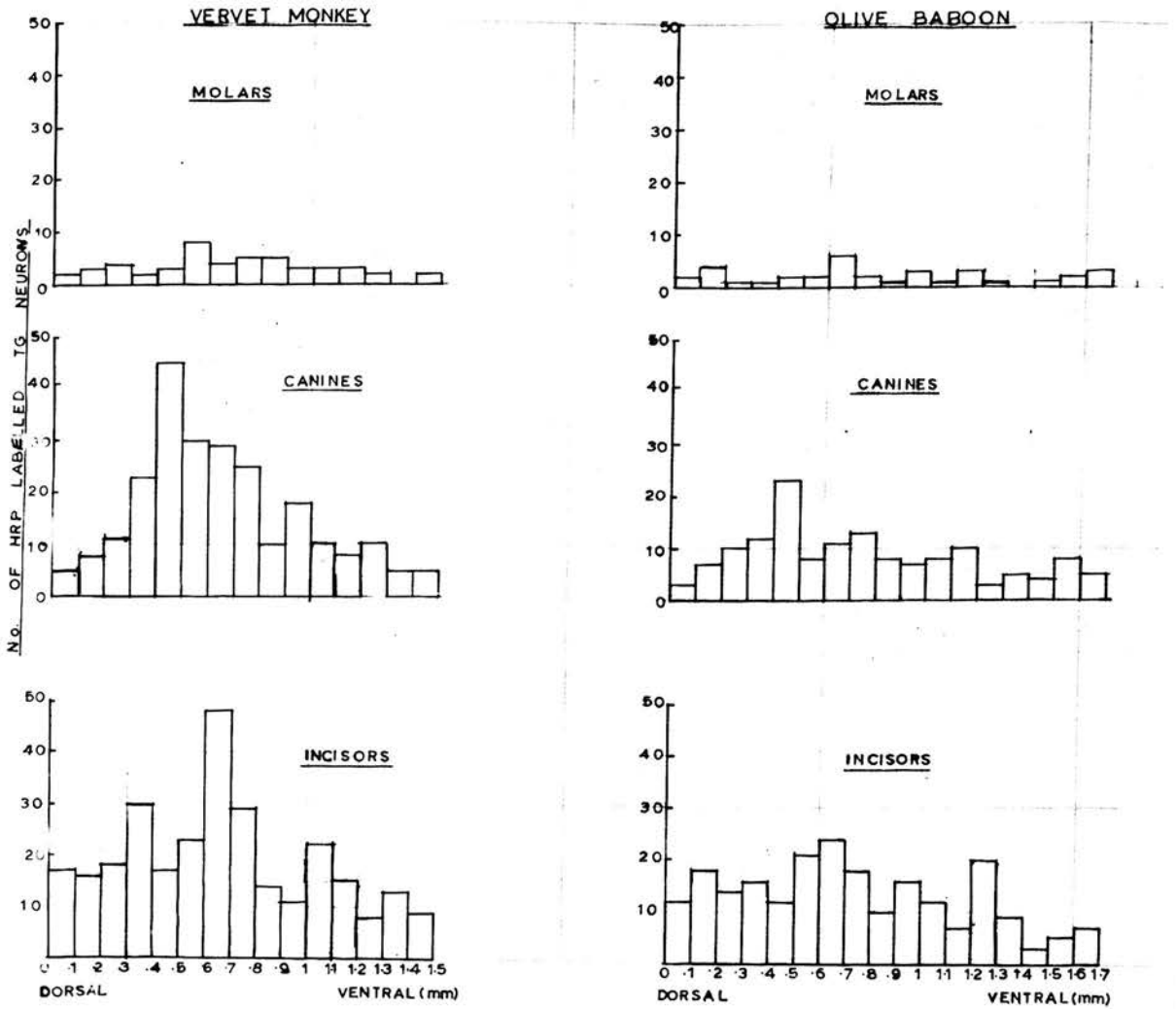


Figure 47. Histograms showing the numerical distribution of the HRP labelled periodontal afferent neurons for incisors, canines and molars along the dorso-ventral extent of the trigeminal ganglion in the vervet monkey and olive baboon.

Table 9 showing the volume density of the HRP labelled neurons ( $V_{V_{LN}}$ ) and the total neurons ( $V_{V_N}$ ) of the trigeminal ganglia of monkeys following HRP application to incisor, canine and molar teeth. Volume percent of the labelled neurons is shown (Vol % LN)

Monkey	Teeth	$V_{V_{LN}}$	$V_V$	Vol % LN
2	Max $I^1 + I^2$	0.004	0.35	1.1
6	Mand $I_1 + I_2$	0.005	0.38	1.3
10	Mand $I_1 + I_2$	0.006	0.36	1.7
5	Mand. C	0.006	0.34	1.7
4(LT TG)	Max. $M^1 M^2$	0.0005	0.35	0.15
9	Max. $M^1 M^2$	0.0008	0.40	0.2



Table 10 showing monkeys and baboons with various teeth types pulp injected with HRP and the number of HRP labelled cells in the trigeminal ganglion.

Animal	Tooth Type	Trigeminal	Ganglion
		RT	LT
Baboon 30	Max. I <sup>1</sup> I <sup>2</sup> (LT)	100	900
Baboon 60	Mand. Dec I <sub>1</sub> I <sub>2</sub> (LT).	-	115
Monkey 7*	Max. Canine (LT)	-	32
Monkey 2*	Mand. M <sub>1</sub> (LT)	-	27
Baboon 33	Mand. M <sub>1</sub> (LT)	-	100

\*Monkeys in which two teeth types were injected with HRP in the same animal.

4.

DISCUSSION

- (A) The quantitative and somatotopic aspects of the afferent connections of the various teeth types to the mesencephalic nucleus of trigeminal nerve and the trigeminal ganglion

4.1 The findings of the present study in the vervet monkey and olive baboon using HRP retrograde tracing method have shown that the primary cell bodies of the proprioceptive periodontal and gingival afferents from the maxillary and mandibular incisors, canines and molars are located mainly in the caudal part of the mesencephalic nucleus of the trigeminal nerve on the ipsilateral side. These findings agree with those of Corbin and Harrison (1940), Jerge (1963a), Cody et al. (1972, 1974), Linden (1978), Gottlieb et al. (1984), in the cat. The present study also shows that the primary cell bodies of general sensory afferents of the periodontal ligament and gingivae of the various teeth types are located preponderantly in the ipsilateral trigeminal ganglion. Kerr and Lysak (1964) and Lende and Poulous (1970) have also shown that the primary cell bodies of the afferents of periodontal ligament in the cat and monkey are found in the trigeminal ganglion. In the present study, the primary cell bodies of the pulpal sensory afferents of the various teeth types are found only in the trigeminal ganglion in agreement with the

findings of Marfurt and Turner (1984) in the rat and with those of Capra et al. (1984), and Byers et al. (1986), in the cat. Thus, the present study shows that the periodontal ligament and gingivae of the monkey and baboon teeth have dual innervation, from the mesencephalic nucleus and the trigeminal ganglion, while the dental pulp is innervated by the trigeminal ganglion.

4.2 The quantitative analysis of the number of labelled mesencephalic neurons innervating the periodontal ligament and gingivae of incisors, canines and molars in the monkey and baboon has shown that the three teeth types are well represented in the mesencephalic nucleus. This suggests that all three teeth types have some functional role in the reflex activity of jaw movements during mastication. The stimulation of the proprioceptors by occlusal contact, the directional sensitivity of the neurons and the single and multitooth mesencephalic units of the various teeth types may be involved in determining whether a reflex response is likely to occur with mechanical stimulation of teeth during mastication.

4.3 The analysis of the differences in the number of labelled mesencephalic neurons for the incisors, canines and molars have shown that the incisors have a higher percentage of labelled mesencephalic neurons than the

canines and molars. Analysis of variance showed that there was no overall significant difference in the number of labelled neurons between the monkey and the baboon as well as between the various teeth types. The difference between the mean number of labelled neurons of the incisors and the canines was less significant than that of incisors and molars using the t-test. Although the sample size was small and the coefficient of variation of the mean was high (30%), the data appears to show that the incisor teeth in the monkey and baboon have slightly higher representation in the mesecephalic nucleus than either the canine or the molar teeth. Byers et al. (1986), has noted a higher density of receptor labelling in the periodontal ligament of incisor and canine teeth in the cat following  $H^3$  Leucine injection to the mesencephalic nucleus. Passatore et al. (1983), have also observed a higher incisor representation than the molar teeth in the mesencephalic nucleus of the rabbit. This may imply that the anterior teeth, and particularly the incisors may have an important functional role in the regulation of mastication.

4.4a The general sensory afferent connections of the periodontal ligament and gingivae to the

trigeminal ganglion have shown that the incisors and canine teeth are well represented in the ganglion compared to the molars which were sparsely represented. The mean number of labelled trigeminal ganglion neurons of the incisors and canines are significantly higher than those of the molars in the monkey and the baboon, using the t-test. Analysis of variance showed that for the labelled trigeminal ganglion neurons, with the teeth types grouped in three, incisors, canines and molars, the F-ratio was very close to the table value. This suggests that although there are no overall significant differences in the number of labelled neurons of the various teeth types there may be differences in pairing. The actual data suggests significant differences in the number of labelled neurons of the incisors and molars. The discrepancy between the t-test and multi-variate analysis may be due to the small sample size and data not being suitable for the analysis of variance. The underlying assumptions in multi-variate analysis may only be approximately valid; for example, the assumption of normal distribution, independent observation and constant variance may not be exact.

4.4b Considering the total number of labelled neurons, the percentage of labelled

neurons of incisors and canines are also higher than that of the molars. The pattern of general sensory innervation showing the incisors, canines to have more neuron connections than the molars found in this study supports the evidence that the anterior part of the oral cavity is more sensitive than the posterior part (Kawamura, 1964; Grossman 1964a, b, Grossman et al., 1965). The afferent fibres of the periodontal ligament which have the cell bodies located in the trigeminal ganglion convey general sensation such as touch, pain and pressure through the mechanoreceptors and free nerve endings (Darian-Smith, 1973). The large representation of the anterior teeth in the trigeminal ganglion may be due to the mucosa, gingivae and the periodontal ligament in the anterior part of the oral cavity having numerous receptors and a high density of nerves and thus being highly sensitive. (Dixon, 1962, 1963b; Grossman, 1964a, b; ; 1967; Grossman et al., 1965, Molnar et al., 1968; Dubner et al., 1978).

4.5 The present study in the monkey and baboon has shown that the periodontal ligament and gingivae of incisors have a significantly high representation in the mesencephalic nucleus and the trigeminal ganglion. The canines are also well represented in the mesencephalic nucleus and in the ganglion. Although in the monkey and baboon, the canine teeth are very prominent and large in size compared to the incisors (Figs. 48a,b) they do not



Figure 48a. Photograph of the head of a male baboon showing the relatively large canines compared to the incisors. . C - Canine, I - Incisors.



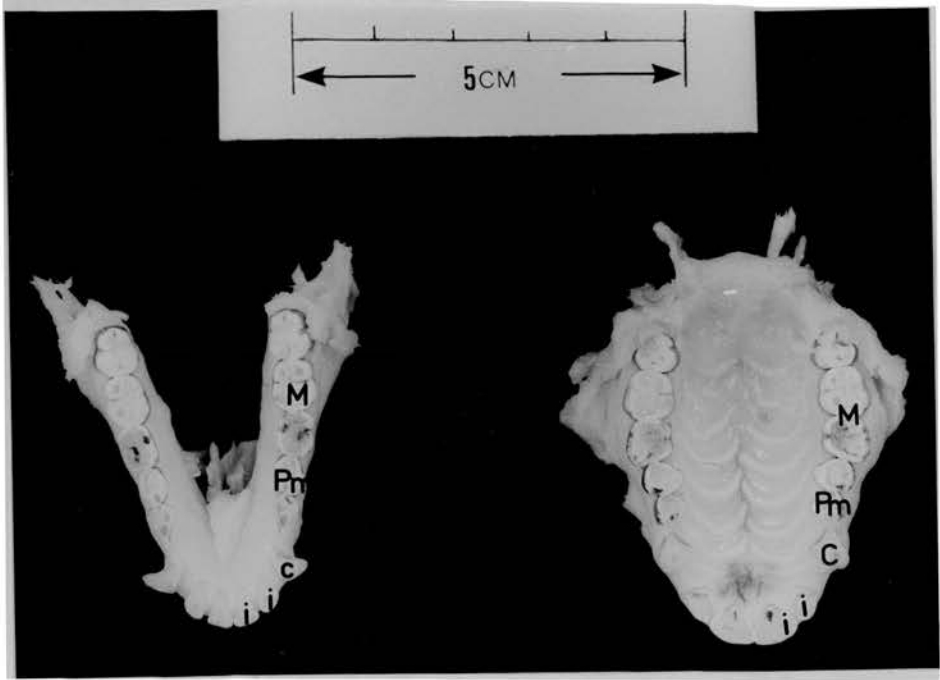


Figure 48b. Photograph of the occlusal view of the maxillary and mandibular teeth of a vervet monkey. x1. Incisor (I) , Canine (C), Premolar (Pm), Molar (M)

appear to be relatively more innervated than the smaller incisors. The posteriorly placed molar teeth which are multirooted and have larger occlusal surfaces compared to the incisal edges of the single rooted incisors (James, 1960, Swindler, 1976), are found to be less well represented in the mesencephalic nucleus and the trigeminal ganglion. Of all the teeth types, the incisors showed the highest neuron count of labelled neurons and the mandibular incisors more than the maxillary incisors. The present study thus shows that the anteriorly placed small incisor teeth have generally a higher neural representation than the prominent canine teeth and the posteriorly placed molars.

4.6 The finding in the present study of a large incisor afferent connection to the trigeminal ganglion and mesencephalic nucleus supports the evidence of low thresholds and high sensitivity of incisors for conscious perception of forces (Manly et al., 1952; Crum and Loiselle, 1972; Owall, 1974). In the newborn rats, neural complexes of the mechanoreceptor type were noted especially in the incisor region (Bonnarire - Mallet and Toux, 1986). The incisor teeth have short roots relative to crown size and incisal surface (Swindler, 1976). This gives a favourable crown/root ratio which may enable a more favourable sensory function with respect to occlusal forces per unit area (Graf, 1974; Lee, 1982). Molars have larger occlusal surface area and are multirooted

teeth (Swindler, 1976). Sensory thresholds and tactile sensibilities of anterior teeth have been found to be greater than that of posterior teeth (Wilkie, 1964, Kawamura, 1967; Manly et al., 1952; Lowenstein and Rathkamp, 1955; Linden, 1975). Lowenstein and Rathkamp (1955) also found that the patient's ability to localize a mechanically stimulated tooth was 100 percent in the anterior teeth but decreased in posterior teeth. Nishiyama et al. (1967), found that the correct judgement to 20 gram load was highest for the incisor and lowest for the second molar and also the judgement was better in localizing stimulation of anterior tooth than the posterior tooth. The touch threshold studies in man has shown that there were differences between the range of forces at which different teeth had their optimum power of discrimination (Bowman and Nakfoor, 1968). Greenberger (1966) investigated the sensitivity of permanent incisors with incompletely formed roots and revealed that the tactile thresholds for young central incisors are lower than those of teeth with completely formed roots. This may be due to root size as the crown/root ratio is thought to be more favourable in the incomplete root raising the possibility of a greater potential for feedback regulation of jaw position.

4.7 The first teeth to erupt into the mouth are the incisors which may establish early subconscious occlusal awareness. During growth of the jaws, the anterior

proprioceptive feedback mechanism may continue to keep the central nervous system informed of how the lower teeth approach the upper teeth during closing motions of the jaw (Lee, 1982). If there is good occlusal relationship of teeth, learned reflexes may then develop by means of which the mandible functions more vertically as the lower teeth approach the upper in final phase of chewing strokes. The proprioceptive feedback mechanism of well related anterior teeth creates a better environment for learning a more vertical and lasting masticatory pattern (Lee, 1982). The afferent connections of the incisors to the mesencephalic nucleus may be important in anterior guidance during the occlusal phase and in reflex jaw movements during mastication. The jaw opening reflex is best elicited by pressure stimulation of gingivae in the anterior part of the mouth, the incisor or canine teeth or the anterior part of hard palate (Sherrington, 1917; Harrison and Corbin, 1942; Jerge, 1964).

4.8 The available evidence suggests that the anterior teeth have mechanical advantage over the posterior teeth in view of the fact that they are situated further from the fulcrum. This position gives them better leverage to offset the closing muscles of mastication. When the average muscle force is 45.5 kg, the occlusal forces are about 32 kg, in molar area and 13.5 kg, in the incisor area (Lee, 1982). It has been argued that because of

unfavourable axial inclinations, the anterior teeth cannot take the loads of mastication and may be loosened in their support tissue. But this is not the case and it is thought that the protective proprioceptive mechanism better protects the anterior teeth from over loading than mechanics alone. This protective mechanism may, however, be diminished with loss or damage of the periodontal ligament and concomitant loss or insensitivity of the periodontal mechanoreceptors (Ramfjord and Ash, 1971; Lee, 1982).

4.9 The periodontal ligament and gingivae of the maxillary and mandibular canines of the monkey and baboon in this study are also found to have large afferent connections to the mesencephalic nucleus and trigeminal ganglion, although less than that of incisors. Studies in the cat have shown that the canine tooth has the higher number of mechanoreceptors (Mei et al., 1975) and is more sensitive to blunt pressure and evoking jaw movements (Corbin and Harrison, 1940; Harrison and Corbin, 1942; Jerge, 1963a; Linden, 1978). Canines were shown to have a high density of receptors of mesencephalic neurons (Byers et al., 1986). Canines in the cat have also been observed to be richly represented in the trigeminal ganglion (Kruger and Michel, 1962; Jerge, 1964). The canine tooth has also been considered to be a "proprioceptive organ" and as such thought to bear the eccentric occlusal load (D'Amico, 1965). In studies in

the cat of discharge properties of neurons in the trigeminal nuclei, a large number of neurons were found to innervate the canine than any other tooth (Eiseman et al., 1963; Kawamura and Nishiyama, 1966). The rich innervation of the cat canine may attribute to the canine the function of reflexly guiding mandibular movements into and out of occlusion (Stern, 1962). In the cat, the canine tooth may be highly innervated as an adaptation for the carnivorous diet, while in monkeys and baboon the canine may not have as essential a role in mastication of mainly fruits, leaves and grass. In the human, Manly et al. (1952), found the threshold for axial forces applied to canines to be higher than that for incisors, although Bonagura et al. (1969), found the maxillary canine to have the highest discrimination ability. A special role for the cuspid tooth in cuspid guided occlusion has been proposed by Hannam et al. (1977), although the precise role of the canine is not clear (Gibbs and Lundeen, 1982).

4.10 Sparse general sensory afferent connections to the trigeminal ganglion from molar teeth was observed in the vervet monkey and olive baboon, while a relatively large proprioceptive connection to the mesencephalic nucleus was noted in this study. However, Byers et al. (1986), showed that in the cat, molars had fewer receptors with the afferent cell body in mesencephalic nucleus although they did not examine all the sections of the periodontal

ligaments of the molars. Corbin and Harrison (1940) failed to evoke responses from mesencephalic root on tactile stimulation of the buccal mucosa in the cat. In the monkey and baboon, the sensory and proprioceptive feedback from the molars may be involved in jaw reflexes at the intercuspil position when large occlusal forces are generated. Functionally, the teeth may be involved in reflexes associated with load bearing forces. The proprioceptive periodontal mechanoreceptor afferents may be involved in peripheral regulation of mastication. During chewing, depending on the food consistency, the muscles may be called upon to contract forcefully to crush the food and maintain contraction or to suddenly stop the contraction when biting on the hard substance which breaks. The sensory feedback from the periodontal ligament on tooth contact or tooth - food - tooth contact may modulate the horizontal jaw reflexes and the unloading reflex (Hannam et al., 1968). The cessation of jaw activity during chewing has also been thought to be due to periodontal feedback (Luschei and Goodwin, 1974, Moller, 1974,). The monkeys and baboons are mainly frugivorous and foliovorous. They also eat nuts, roots and shrubs, and are thought to be omnivorous as they occasionally eat insects as well (Post et al., 1978 a,b). The general sensory and proprioceptive afferent connections of the molar teeth may be functionally involved in the conscious appreciation of forces on the



molar teeth generated in leaf, root and shrub crushing and grinding. In addition the afferents may also be involved in positioning of the food bolus between the teeth (Appenteng et al., 1982).

4.11 In the present study in two Cercopithecine primates, the labelled periodontal mesencephalic afferents neurons of incisors accounted for about 6.4%, canines and molars 4.4% and 4.5% respectively, while the maxillary teeth accounted for 6% and mandibular teeth 9.2% of the <sup>total</sup> mesencephalic neurons unilaterally. In the baboon, labelled periodontal mesencephalic afferent neurons of incisors, canines and molars accounted for 4.5%, 3.4% and 2.5% respectively while the maxillary teeth accounted for 4.8% and mandibular teeth 5.6% of the total mesencephalic neurons unilaterally. Gottlieb et al. (1984), found that in the cat, 7.3% of the mesencephalic neurons innervate the anterior mandibular teeth while about 12% innervate the mandibular posterior teeth. They found 16.8% of maxillary representation from infraorbital nerve. Bosley et al. (1983), found 13.5% of the cat mandibular teeth periodontal mesencephalic afferent neurons located in the mesencephalic nucleus. In the cat, the mandibular teeth and particularly the canine have been found to have the largest number of mesencephalic units represented; then the mandibular molars and least the mandibular incisors (Linden 1978). However, Passatore et al. (1983), found in the rabbit that, of the

periodontal mesencephalic neurons, incisors showed the highest representation of 40%, inter-alveolar gingivae 29% and molars 12%.

4.12 The differences in the mesencephalic neuron representation of the various teeth types in the cat, rabbit and the primate, may be a reflection of the different masticatory behaviour. In the primate, the anterior and posterior teeth are important in all functional movements of the jaw during mastication. In the rodent, the anterior teeth and in the cat, the canines and mandibular teeth may be more important for the sensory feedback from these teeth in regulating the masticatory movements of the jaw. In the primate, the functional role of incisors in anterior guidance of occlusion and sensory and proprioceptive role in mastication and protective reflexes has been discussed (Lee, 1982). The role of canine in cuspid guidance in occlusion has been emphasized (Hannam et al. 1977). In the monkey and baboon, the prominent canines may be used more in offence and defence and thus is not as heavily innervated as the incisors. Molars are load bearing teeth, being used mainly for crushing and grinding the food. The role of molar teeth in cuspal guidance and tooth contacts during chewing reflex jaw movements has been considered (Anderson 1976). Heath (1948) recorded incisor bite pressures of between 1/3rd and 1/2 of those of molars. Gibbs (1971)

suggests that molar teeth at closure provide protection for both the temporomandibular joint condyles and prevent them from accepting elevating forces, thus providing concept of molar protected temporomandibular joint.

4.13 The present study has attempted to quantify the trigeminal ganglion neurons innervating the various teeth types in the monkey and baboon. Although a somatotopic distribution of the periodontal sensory afferent neurons in the trigeminal ganglion has been known (Darian-Smith 1973), no quantification of the number of trigeminal ganglion neurons innervating the various teeth types has been carried out in other species. Gonzalo-Sanz and Insuasti (1980) noted a large number of labelled neurons in the trigeminal ganglion as well as ipsilateral mesencephalic nucleus of trigeminal nerve in the rat following HRP application to the maxillary nerve. Gottlieb et al. (1984), also noted in the cat that HRP labelled neurons were found in the ipsilateral trigeminal ganglion following HRP injection into the periodontal ligament and application of HRP to inferior alveolar nerve and infraorbital nerve. Both these studies did not quantify the labelled trigeminal ganglion neurons. Some indications as to the neuron density within the trigeminal ganglion somatotopically representing the various branches of the trigeminal nerve are given in the HRP study of Marfurt (1981a). Gregg and Dixon (1973) have quantified the neurons in the rat for various

branches of the trigeminal nerve. They expressed the percentage of the chromatolytic neuron for the individual branches. In the physiological study, Lende and Polous (1970) have expressed the percentage of the units responding to general sensation, pain, pressure stimulation and jaw movements.

4.14 The afferent connections of the dental pulps of the various teeth types to the trigeminal ganglion have been shown in the present study in the monkey and baboon. Incisors appear to have a large number (900 cells, baboon; 115 cells, monkey) of neurons innervating the pulp, while fewer neurons were noted for the molar (100 cells, baboon and 27 cells, monkey). The maxillary canine in one monkey showed a rather small number (32) of neurons labelled following HRP application to tooth pulp. However, the cement was noted to have been displaced due to poor retention and thus dilution of HRP may have occurred. The maxillary incisors in an adult male baboon have shown a very high representation, (900 cells) in the ipsilateral ganglion as well as 100 cells in the contralateral ganglion. There appears to be no strict somatotopic position of labelled neurons of the maxillary incisor pulps within the maxillary region as about 50 to 70 labelled neurons were observed in the ophthalmic region on the ipsilateral side. Lack of strict somatotopy has also been shown by Henry et al. (1986), in the rat. The large number of neurons innervating the

incisors may account for the greater sensitivity of incisors as shown by low threshold. It is known that pulpal afferents may be branched (Lisney and Mathews, 1978), and thus some of the labelled ganglion neurons may innervate more than one tooth.

4.15 Chiego et al. (1980), also studied nerve supply to primate teeth using HRP method, but no quantification of pulpal afferent labelled trigeminal ganglion neurons was carried out. In the rat, Aker and Reith (1981) examined the trigeminal ganglion neurons innervating the mandibular molar tooth and found 185-318 labelled cells which account for almost 0.5-0.7% of the total ganglion neurons, 23,000-46,000, (Aldskogius and Arvidsson, 1978). Arvidsson (1975) in the cat, found more labelled neurons for the maxillary canine (136 cells) than mandibular canine (28 cells). Fuller et al. (1979), found 252 labelled cells following HRP application to the lower canine in the cat. Wilson et al. (1983), found that in the cat, the <sup>lower</sup> central incisor pulp had 5-21 labelled neurons, 1st lateral incisor, 10-83 and 2nd lateral incisor 16-23. The fewer number of neurons for the incisor as compared to the canines is suggested to be due to smaller size of the incisors. It may also be due to the more importance of canine in sensory feedback in the cat. However, there was a very marked individual variation of the number of labelled neurons for similar teeth in these studies which may depend on the depth of

the cavity and other factors discussed more fully in the technical consideration of the HRP method. Using HRP method, in the primates, Kubota et al. (1979), have shown that in addition to the trigeminal ganglia, the sympathetic cervical ganglia also innervate the teeth pulp. On HRP application to exposed molar tooth pulp, a few labelled cells were found in the ipsilateral trigeminal ganglion. For the upper molar, 2-20 trigeminal ganglion cells were labelled while for the lower molar, a few labelled cells (no number given) were seen (Kubota et al., 1979). In the present study, for the lower first molar tooth pulp of the monkey and baboon, relatively few labelled neurons were observed compared to the incisors.

4.16 No labelled mesencephalic neurons were observed following HRP application to the pulp in the present study. The findings agree with those of Kubota et al. (1979), in the primates. However Chiego et al. (1980), found some labelled mesencephalic neurons, which may be as a result of leakage of the tracer from the pulp to the periapical region which is rich in proprioceptors and nerve endings (Byers and Mathews, 1977; Kubota and Osnai, 1977; Byers et al., 1986). Capra et al. (1984), and Marfurt and Turner (1984) did not observe any labelled mesencephalic neurons innervating the dental pulp in the cat and rat respectively.

4.17 The trigeminal ganglionic pulpal afferent neurons are involved in subserving pain sensation, pulpal reflexes and regulation of dentine formation (Avery, 1981; Gunji, 1982). Although the sensation elucidated from the tooth pulp has been presumed to be pain only, it has been thought that the tooth pulp may subserve tactile information (Dubner et al., 1978; Byers et al., 1982). Central projections from tooth pulp afferents to the main sensory and subnucleus oralis has been noted (Marfurt, 1981b). Presence of corpuscular nerve endings in pulp (Pimenidis and Hinds, 1977) and the pressoreceptive study of the Lowenstein and Rathkamp (1955) showing higher thresholds for pulpless teeth seem to suggest a tactile mechanoreceptive function in pulp. However, Stewart (1927) and Linden (1975) found no difference in touch thresholds of vital and non-vital human teeth. Dubner et al. (1978), have reviewed the evidence regarding tooth pulp sensations and suggest that natural means of tooth pulp stimulation during experimental procedures is called for since there is doubt that pain is the only sensation that can be elicited from tooth pulp. Chatrian (1982) studied the qualities of sensations following tooth pulp electrical stimulation in human and used questionnaires. They found that non-painful sensations can rise from electrical stimulation of pulp not involving the periodontal ligament or gingivae and suggest that tooth pulp afferents may have some unspecified sensory function



besides pain perception, Dong et al. (1985), showed that some intradentinal receptors in canine teeth of the cat detect mechanical transients applied to intact enamel suggesting that dental innervation may play a non-nociceptive role in oral function such as detecting tooth contact during mastication and swallowing. However, Matthews (1986) found no evidence of intradentinal receptors responding to mechanical stimulation of enamel.

4.18 Pulpal reflexes and central connections are quite complex and the precise role that the higher centres and brainstem nuclei play in pulpal sensation is not clear (Matthews et al., 1976; Bratzlavsky et al., 1976; (人 Dubner et al., 1978). Interaction of tooth pulpal and periodontal ligament receptors in the cat, dog and monkey have been shown (Anderson and Mahan, 1971; Mahan and Anderson, 1971).

4.19 The sample size for the study of pulpal connections in the present study was small. Also injections of pulp were made in the monkey where periodontal ligament injections were made in the opposing jaw in other tooth type. This may have influenced the results. Further, well controlled studies of pulpal connections are required where the cavity depth is controlled and any leakage of the tracer from the pulp to periapical region is determined by sectioning the tooth after the experimental procedure.

4.20 The somatotopic distribution of the mesencephalic periodontal afferents from the various teeth types was found to be strictly in the ipsilateral mesencephalic nucleus in the monkey and baboon. The periodontal afferent neurons of the various teeth types were mainly located in the caudal part of the nucleus, but there was no somatotopic organization for the individual teeth types in this region. The ipsilateral connections of the mesencephalic afferent neurons to the teeth have been noted by others (Corbin and Harrison, 1940; Gottlieb et al., 1984; Capra et al., 1984; Byers et al., 1986). Most of the studies of muscle spindle afferents have also shown an ipsilateral mesencephalic connection in the monkey, rat and cat (Ibrahim and Leong, 1979; Jacquin et al., 1983 a, b; Walberg, 1984; Capra et al., 1985, Rokx et al., 1986 a). In the electrophysiological study in cat, rabbit and monkey (Smith et al., 1967; Dault and Smith, 1969; Passatore et al., 1983) and degeneration study in rat (Foster, 1973), contralateral mesencephalic connections have been noted.

4.21 The afferents from the periodontal ligament to the trigeminal ganglion were preponderantly ipsilateral. In two animals out of four, where HRP had been injected into the periodontal ligament of maxillary incisors, some labelled neurons were observed in the contralateral trigeminal ganglion. There was no labelling in the contralateral ganglion following HRP injection into the

periodontal ligaments of maxillary canines and molars. In the mandibular incisor there was labelling in the contralateral ganglion in 3 animals out of 4. Thus in the mid-line region, there may be overlap of some nerves from the contralateral side or the HRP may have spread to the adjacent gingivae across the midline. For the tooth pulp, there was labelling in the contralateral trigeminal ganglion in the baboon following HRP injection into the tooth pulp of maxillary incisors. The nerve plexus innervating the maxillary incisor pulps may have some contribution from the contralateral side. The maxillary incisor periodontal ligament may also have some contribution from the contralateral side or the HRP may have spread to the gingivae across the midline. The evidence regarding the innervation of anterior teeth, namely incisors and canines from contralateral as well as ipsilateral ganglion has been provided by Anderson and Pearl (1974a,b), Pearl et al. (1977); Shellhammer et al. 1984). Others have shown an ipsilateral connection only (Arvidson, 1975; <sup>Fuller and Winfrey, (1980)</sup>Wilson et al., 1983). In most of the studies in the rat and cat, using the method of HRP application to cut branches of trigeminal nerve and to tooth pulp, an ipsilateral connection has been shown (Aker and Reith, 1981; Marfurt, 1981b and Gottlieb et al., 1984). In the primate, Chiego et al. (1980), found some contralateral trigeminal ganglion neurons following injection to mandibular premolars, although the average

grain density of the label was much less in the contralateral ganglion neurons. Thus it appears that the afferent general sensory connections of the teeth to the trigeminal ganglion are mainly ipsilateral. There may be some slight overlap in the midline, but the findings are not consistent.

4.22 The topographic and cytoarchitectural organization of the brainstem of the vervet monkey and olive baboon was found to be similar to that described by Feremutsch (1965) and Gerhard and Olzeweski (1969). The structural organization of the mesencephalic nucleus of trigeminal nerve in the monkey and baboon was similar. The general distribution and morphology of the mesencephalic neurons was in agreement with that described by Weinberg (1928), Sheinin (1930) and Sivanandasingham and Warwick (1976) in monkeys and dogs. There was an increase in the density of cell from the level of the posterior commissure to the inferior colliculi with some regions of sparscity in the caudal regions of inferior colliculi. In the rostral pons, there was an increase in cell density. The feature of cell clustering was observed in the monkey and baboon as also observed by Weinberg (1928) in the monkey and Sivanandasingham and Warwick (1976) in the rhesus monkey. In the cat, the arrangement of cells is similar to that described in the monkey and baboon (Weinberg, 1928; Sivanandasingham and Warwick, 1976; Brodal and Saugstad, 1965; Nomura et al., 1985; Capra et al., 1985). The

arrangement of neurons in the rat, however is different in that there is higher density of cells in the caudal part of the nucleus where 60% of the cells are found (Weinberg, 1928; Hinrichsen and Larramendi, 1969; Rokx et al., 1986a).

4.23 The findings of the present study shows that the distribution of HRP labelled periodontal afferents from incisors, canines and molars are located mainly in the caudal part of the ipsilateral mesencephalic nucleus of the trigeminal nerve. Of the total labelled mesencephalic neurons for the three teeth types, about 80-90% were located in the caudal part of the nucleus at the level of the inferior colliculi and pons. Labelling was very sparse (about 12%), in the rostral part of the nucleus at the level of the superior colliculi. The caudally labelled neurons were large and small, oval or round as well as fusiform or bipolar and multipolar. The labelled neuron was either single or two neuron seen adjacent to other non-labelled cells. These observations in the monkey and baboon agree with the anatomical findings (Gottlieb et al., 1982, 84; Bosley et al., 1983; Capra et al., 1984; Byers et al., 1986) and physiological findings (Corbin and Harrison, 1940, Cody et al., 1974; Linden 1978) in the cat. Similar observations of mainly caudal distribution of periodontal afferents have been made in the rat (Gonzalo-Sanz and Insuasti, 1980; Matesz, 1981; Jacquin et al., 1983a). In the rabbit, Passatore et al.

(1983), also showed a caudal location of 85% of the periodontal proprioceptive afferent units.

4.24 Functionally, mesencephalic neurons subserve proprioceptive modalities from the oro-facial region. Proprioceptive afferents of the jaw-closing muscle spindles are located along the rostro-caudal extent of the mesencephalic nucleus in the cat, rat, rabbit and monkey (Ibrahim and Leong, 1970; Walberg, 1984; Capra et al., 1985; Jacquin et al., 1983ab). About 70% of the mesencephalic neurons innervate the stretch receptors of muscles while 20-30% innervate teeth (Corbin, 1940. Ibrahim and Leong, 1979; Passatore et al., 1983; Jacquin et al., 1983b; Walberg, 1984; Gottlieb et al., 1984; Capra et al., 1985; Nomura et al., 1985). Evidence for location of the proprioceptive afferents from extraocular muscles in the mesencephalic nucleus is conflicting (Fillenz, 1955; Cody et al., 1972). Sherif et al. (1981), found 4-10% of fibres of mesencephalic neurons in the trochlear nerve of <sup>the</sup> cat. In the cat and monkey, the soma of extraocular muscles have been found in the trigeminal ganglion (Porter and Spencer, 1982; Porter et al., 1983). However, Lucifer and Eggezi (1986) found a few labelled mesencephalic neurons following HRP application to ethmoidal nerve in the cat and suggest that these could be afferent neurons of proprioceptors of the nose or extraocular muscles. Tooth pulp afferent neurons were not observed in the mesencephalic nucleus in

the present study and by Capra et al. (1984), Byers et al. (1986), although Chiego et al. (1980), found some labelled pulpal mesencephalic afferent neurons in the monkey .

4.25 The functional significance of the caudal aggregation of the mesencephalic periodontal afferents have been debated. Marini and Bortolami (1982) for instance, found that in the frog, the mesencephalic neurons responding to jaw closing muscles are represented in the dorsal part whereas the jaw opening ones are located more ventrally. Passatore et al. (1983), in the rabbit showed that stimulation of caudally placed periodontal afferent neurons produced jaw opening movements.

4.26 The caudal location of the periodontal mesencephalic neurons and their possible connections have been discussed by Gottlieb et al. (1984). Mesencephalic connections to the cerebellum have been indicated in several species and man (Pearson, 1949; Brodal and Saugstad, 1965. Bortolami et al., 1972; Roberts and Witkovsky, 1975). Chan-Palay (1977) has also shown mesencephalic projections to the cerebellum. The caudally placed periodontal mesencephalic neurons have been thought to be favourably placed to send projections via brachium conjunctivum to the cerebellum. Marfurt (1981b) also noted that the central processes of the



periodontal mesencephalic neurons project to the cerebellum via the superior cerebellar peduncle. Taylor and Elias (1984) have studied the interaction of periodontal and jaw-elevator spindle afferents in the cerebellum and found evidence of direct mossy fibre collateral projection to the anterior lobe from periodontal afferents only. Muscle spindle afferents do not show such a projection. Elias et al. (1985), provided further evidence for direct periodontal mesencephalic afferents to the cerebellar cortex in the ferret. The cerebellar projection may be involved in sensory calibration of jaw movements (Taylor and Elias, 1984).

4.27 The cerebellum does not initiate any masticatory movements but may be concerned with synergism of the muscles of mastication. This influence may not be critical since an individual with cerebellar damage is capable of chewing food fairly well (Kawamura, 1964). However, cerebellar disorders such as asynergy would impede mastication (Mercurio, 1981). The mesencephalic cerebellar projection may be a part of the mechanism that controls the force of the bite (Brodal, 1981).

4.28 The location of the cell bodies of the proprioceptive afferents from the muscles of mastication and teeth in mesencephalic nucleus within the central nervous system makes it unique and indicates that the

motor control of jaw activity is specialized (Luschei and Goldberg 1982; Taylor and Appenteng, 1981; Taylor, 1981). Another interesting parallel of the teeth afferents with muscles afferents is that muscles also have the afferent cell bodies located in the trigeminal ganglion and mesencephalic nucleus (Darian-Smith, 1973; Ibrahim and Leong, 1979). Lende and Poulous (1970) found 3% of the trigeminal ganglion units responding to jaw movements. The jaw-closing mesencephalic muscle afferents make monosynaptic reflex connections with the jaw-closing moto-neurons eliciting the jaw-jerk reflex. On tooth contact the periodontal ligament receptors are activated and elicit polysynaptic jaw-opening reflex such that the jaw-closing muscle motor neurons are inhibited and the jaw-opening moto-neurons has excitatory action (Sumino, 1976). The jaw-opening reflex elicited by peripheral stimulation of dental mechanoreceptors or peripheral nerves does not allow discrimination between the mechanoreceptors whose afferent neurons are located in the trigeminal ganglion (the central fibres project to the main sensory and subnucleus oralis) and those located in the mesencephalic nucleus. The more numerous trigeminal ganglion neurons are capable of eliciting reflex jaw-opening. Nevertheless, the periodontal mesencephalic afferents participate in jaw-opening although located along with muscle spindle afferents which elicit jaw-closing reflex. (Hannam, 1976; Passatore

et al., 1983). Very few muscle spindles have been observed in the jaw-opening muscles (Dubner et al., 1978). No monosynaptic periodontal mesencephalic neuron connections with jaw opening motor neurons have been observed (Marfurt, 1981b). However, recently muscle spindles have been identified in the guinea-pig lateral pterygoid muscle and monosynaptic reflex connections observed (Nozaki et al., 1986). On injecting HRP into the lateral pterygoid muscle, 15-20 labelled mesencephalic neurons were observed ipsilaterally in the caudal part of the nucleus. Masseter muscle HRP injection labelled 174-228 mesencephalic neurons (Nozaki et al., 1986).

4.29 In the baboon and vervet monkey, clusters of mesencephalic neurons of 2-9 cells were present all along the rostro-caudal extent of the nucleus. Large clusters or aggregation of mesencephalic neurons were common in the caudal part of the nucleus where the periodontal afferents were predominant. Some of the cells of the cluster showed soma-soma contact with the cell membranes in a gap junction with maculae adherens (Hinrichsen and Larramendi, 1970; Peters et al., 1976). Cluster formation with soma-soma and axo-somatic contact and gap junctions in mesencephalic nucleus has been observed in many species (Weinberg, 1928; Hinrichsen and Larramendi, 1968; 1970; Sivanandasingham and Warwick, 1976; Gottlieb et al., 1984; Nomura et al., 1985; Rokx et al., 1986a).

Electrotonic coupling of mesencephalic neurons has been demonstrated (Baker and Llinas 1971, Hinrichsen, 1976) as well as synapses have been noted in the mesencephalic neurons (Hinrichsen, 1976, Nomura et al., 1985). The mesencephalic neuron synapses which may be excitatory or inhibitory may serve to alter the degree of coupling between cells (Spira and Bennet, 1972).

4.30 Electrotonic coupling has not been found to be more in the caudal part of the mesencephalic nucleus (Hinrichsen, 1970, Baker and Llinas, 1971). Linden (1978) did not find any mesencephalic neuron which responded to both, jaw opening and jaw-closing. Electrotonic coupling between muscle spindle cells has been suggested by Hinrichsen (1976). Passatore et al. (1983), in the rabbit, noted many gap junctions in the mesencephalic neurons in region with mixed, periodontal and muscle spindle mesencephalic neurons. They suggest that coupling may occur between these cells. Anatomic studies have shown one or two labelled cells in a cluster (present study; Gottlieb et al., 1984; Capra et al., 1984;) following HRP application to the periodontal ligament. Capra et al. (1985), also observed one or two labelled cells of masseter and temporalis afferents in a cluster supporting the evidence that single clusters may supply different masticatory muscles and teeth and that such association may form a functional unit. Thus, activity occurring in a single cell of the unit may

trigger or facilitate activation of the entire unit. If electrotonic coupling exists between muscle afferent cells, it could modify the monosynaptic response to lengthening of jaw-closing muscles. (Henrichsen, 1976) Electrotonic coupling may be present in mesencephalic neurons of dental afferents in the jaw-snap reflex produced by tooth tapping in the selachian (Roberts and Witkovsky, 1975).

4.31 The **segregation** of periodontal mesencephalic afferent neurons of the various teeth types in the caudal part, and the possible electrotonic coupling between the different periodontal and/or muscle spindle neurons may allow for the synchronization of the dental input and mediation of the jaw reflexes during mastication. The mesencephalic neurons demonstrate fine force and directional sensitivity and some are rapidly adapting and others slowly adapting (Linden 1978). This type of nerve distribution would provide extremely refined inputs to the central nervous system for sensory motor control of oral behaviour (Taylor and Appenteng, 1981; Taylor, 1981; Luschei and Goldberg, 1982).

4.32 In the caudal pontine region in the monkey and baboon smaller and often faintly labelled mesencephalic neurons were observed ipsilaterally. Walberg (1984) also noted faintly labelled multipolar mesencephalic neurons ipsilaterally in the pontine part of the nucleus

following HRP injections to the muscles. Ramon y Cajal (1909) noted multipolar mesencephalic neurons in the caudal pontine region and considered the multipolar cells as displaced locus coeruleus neurons. Walberg (1984) debates whether the multipolar locus coeruleus cells are functionally different from the mesencephalic cells. In vitro study of electro-physiological properties of neurons contained in locus coeruleus and mesencephalic nucleus of trigeminal nerve has shown some differences and intracellular injection of lucifer yellow revealed that the cell bodies of locus coeruleus were small and multipolar whereas mesencephalic neurons were larger and generally monopolar cell bodies (Henderson et al. 1982). Multipolar mesencephalic neurons were also observed by Gottlieb et. al. 1984; Capra et al. (1985), and Nomura et al., (1985). The axons of these faintly labelled cells may branch extensively. The connections of the multipolar cells may be complex and may project to the cerebellum (Saigal et al., 1980; Elias et al., 1985).

4.33 The HRP findings of the present study show that in the caudal pontine region of the mesencephalic nucleus, in addition to the ipsilaterally labelled mesencephalic neurons, some "faintly labelled" neurons are observed bilaterally following HRP injection to the periodontal ligaments of teeth on the ipsilateral side. It is suggested that the "faintly labelled" bilateral neurons may be the interneurons of the supratrigeminal nucleus.

An aggregation of neurons ventro-lateral to the locus coeruleus and adjacent to the motor nucleus is thought to be the supratrigeminal nucleus although there is no description of the supratrigeminal nucleus in the primate pons and medulla (Gerhard and Olszewski, 1969). The supratrigeminal nucleus is located around the nucleus subcoeruleus which lies across from the locus coeruleus. The supratrigeminal nucleus has been described in the rat (Mateusz, 1980; Jacquin et al., 1983a; Rokx et al., 1986b) and the cat (Jerge 1963b, 1964 Kawamura, 1974), in the equivalent position as observed in the monkey and baboon. These interneurons of the supratrigeminal nucleus are thought to receive collateral connection from the mesencephalic nucleus and sensory ganglion and have a possible proprioceptive function in jaw-opening and jaw-closing reflexes (Jerge, 1963b, 1964; Kidokoro et al., 1968; Kawamura, 1974; Sumino, 1971, 1976; Dubner et al., 1978, Rokx et al., 1986b). Jacquin et al. (1983 a), in the rat observed some supratrigeminal neurons labelled following HRP application to the whole mandibular nerve, inferior alveolar nerve and motor root and suggest that these neurons may be associated with mesencephalic neurons. Gonzales-Sanz and Insuasti (1980) have reported labelled supratrigeminal neurons following HRP application to the maxillary nerve in the rat. Jerge (1963b) recorded units from the supratrigeminal nucleus and suggest that these neurons may innervate periodontal



ligament and thus participate in the control of mastication. Matesz (1981), using cobalt labelling technique in the rat observed collaterals to the supratrigeminal nucleus consisting of medium sized neurons.

4.34 However, the bilaterally observed supratrigeminal neurons in the pons could also be cells of the locus **coeruleus** which often has brown granules present and is found bilaterally. The appearance of the brown granules in the region of the locus coeruleus in the frozen and paraffin wax sections was not consistent. The difference in the density of "labelled cells" in the TMB processed sections as compared to the unprocessed sections from the same series indicated that there were HRP faintly labelled neurons in the caudal part of the mesencephalic nucleus in the region of supratrigeminal nucleus. Thus it is likely that the HRP was transported along the collateral from the ipsilateral mesencephalic neurons and then to the contralateral supratrigeminal neurons. It may also have been transported along the central process of the ipsilateral trigeminal ganglion neurons to the interneurons of the supratrigeminal nucleus bilaterally. Marfurt (1981b), and Jaquin et al. (1983b), have shown the central connections of the trigeminal ganglion to the brainstem trigeminal nuclear complex using free HRP-Sigma VI. Some of the the faintly labelled multipolar mesencephalic neurons observed in the caudal pontine

region by Walberg (1984) may possibly be the supratrigeminal neurons.

4.35 Thus it appears that three cell types may be intermingled in the caudal pontine region of the mesencephalic nucleus. These may be cells of locus coeruleus, the smaller multipolar mesencephalic neurons and cells of supratrigeminal nucleus which could be interneurons (Ramon Y Cajal, 1909; Foster 1973; Dault and Smith, 1979; Walberg, 1984; Rokx et al., 1986a,b).

4.36 The action of the jaw-closing and jaw opening muscles on the two sides is generally co-ordinated. There is conflicting evidence regarding the bilateral mesencephalic projection (Smith et al., 1967, 1968). However, it is possible that the caudal supratrigeminal neurons may have been stimulated in these experimental procedures. Dault and Smith (1979) have suggested that 15% of the mesencephalic neurons are interneurons. Smith et al., (1967), have discussed the bilateral integrating system for muscles of mastication in <sup>the</sup> monkey and cat and the close association of the mesencephalic nuclei to such a system. Contralateral inhibition of masseteric muscle following stretching of the ipsilateral muscle does occur which Kawamura (1970) suggests is mediated by a pathway including interneurons within the supratrigeminal nucleus. Nakamura et al. (1973 a, b), and Rokx et al.

(1986b) have also discussed the inhibitory pathway through the interneurons of supratrigeminal nucleus.

4.37 The present study indicates a possible bilateral connection to the supratrigeminal nucleus from periodontal afferents while the input to the mesencephalic nucleus and the trigeminal ganglion is ipsilateral. The functional role of the supratrigeminal nucleus as a pool of interneurons in modulating the bilateral control of oral motor behaviour is considered (Rokx et al., 1986b). Mesencephalic nucleus connections to other reticular formation regions such as the nucleus of Probst and parvocellular zone have also been shown (Ruggerio et al., 1982). Thus the proprioceptive input from one side may be mediated polysynaptically to the **bilateral** oral final pathway for motor function along these interneurons.

4.38 The structural organization of the trigeminal ganglion of the vervet monkey and olive baboon was similar except for the difference in size. The ganglion of the baboon is about twice the size of the ganglion of the monkey. The topography of the ganglion of the monkey and baboon was similar to that described by Hill (1966) and Gasser and Wise (1972). The microscopic organization of the ganglion neurons was similar to that described for the cranial ganglia (Lieberman, 1976). The ganglion neurons were arranged as a crescent shaped band extending

from the lateral aspect where the mandibular nerve branches across the middle maxillary region to the medial ophthalmic part.

4.39 The soma of the trigeminal ganglion neurons innervating the mandibular teeth were located mainly in the postero-lateral aspect of the ganglion while those of the maxillary teeth were located in the middle part of the ganglion. The findings of this study shows that the trigeminal ganglion in the vervet monkey and olive baboon is generally somatotopically organized. The ganglion neurons that innervate the mandibular anterior as well as posterior teeth were found along the dorso-ventral extent of the ganglion in the mandibular compartment. The anterior teeth showed the largest concentration in the middle part of the ganglion along the dorso<sup>o</sup>ventral extent of the ganglion with a concentration of labelled neurons in the centre. The findings of the present study generally agree with those of Gregg and Dixon (1973) and Jacquin et al., (1983a) in the rat and of Kerr and Lysak (1964), Beardeau and Jerge (1968), Marfurt (1981a) and Lende and Poulous (1970) in the cat and monkey. These studies showed that the soma of the mandibular nerve which innervates the lower third of the face and ventral half of the oral cavity are distributed within the postero-lateral extent of the ganglion in the mandibular compartment. The soma of the maxillary nerve that innervate the middle part of the face and dorsal part of

the oral cavity are found in the middle part of the ganglion. The cell bodies of the ophthalmic branches supplying the dorsal third of the face are located antero-medially (Marfurt 1981a; Lucifer and Eggezi 1985).

4.40 However there is some conflicting evidence as to the dorso-ventral somatotopy. Kerr and Lysak (1964) and Lende and Poulous (1970) noted no dorso-ventral somatotopy while Gregg and Dixon (1973) and Marfurt (1981a) found evidence of somatotopy of the anterior part of the face on the ventral aspect of the ganglion and posterior region in the dorsal aspect of the ganglion.

4.41 HRP labelled cells of the mandibular and maxillary teeth were generally dispersed within the mandibular and maxillary compartment respectively. There was no somatotopic organisation within the mandibular and maxillary compartments for incisor, canine and molars. These findings also agree with the electrophysiological study of Lende and Poulous (1970) in the monkey that there was no indication of spatial localization of trigeminal ganglion neurons according to the territories of the mandibular division nerve branches. Jacquin et al. (1983a), also noted that HRP labelled inferior alveolar nerve soma occupied the entire dorsoventral extent. Capra et al. (1984), noted labelled ganglion neurons within the linearly arranged clusters of neurons.

4.42 There was no marked regional overlap of periodontal afferent trigeminal ganglion neurons of the mandibular teeth and maxillary teeth. However, the afferent neurons of the maxillary incisor tooth pulp in one of the baboons were seen to be in the ophthalmic region in the ventral and middle part of the ganglion.

4.43 The present anatomical study using the method of HRP axonal retrograde transport has attempted to show the afferent neurons of trigeminal ganglion and mesencephalic nucleus innervating the periodontal ligaments and tooth pulps of various teeth types in the vervet monkey and olive baboon. However, it has been shown that single nerve fibres collateralize to innervate adjacent teeth (Sakada and Kamio, 1971; Jones et al., 1984). Capra et al. (1984), showed that the tooth pulp is innervated by trigeminal ganglion neurons only, while the periodontal ligament and gingivae are innervated by trigeminal ganglion and mesencephalic neurons and they also showed that the same trigeminal ganglion neuron does not innervate pulp and periodontal ligament. This is in agreement with physiological observations (Greenwood 1973; Matthews, 1977). Tooth pulp receives a discrete innervation and branching pulpal afferents bypass non-pulpal targets. Electrophysiological studies have also shown single and multitooth trigeminal and mesencephalic units (Linden 1978; Appenteng et al., 1982). If one neuron sends branching collaterals to adjacent teeth and

gingivae it is likely that the same neuron may be retrogradely labelled from branching peripheral collaterals in the periodontal ligaments of the different teeth types. In addition, some other limitations of the HRP method needs to be considered.

B) Technical Considerations of the HRP method

4.44 The quantitative aspects of the neural connections of teeth to the trigeminal ganglion and the mesencephalic nucleus found in the present study need to be considered in the light of some technical limitations of the HRP method. One of the common findings by many investigators using the HRP method has been the variability of the number of labelled neurons following similar experimental procedure and survival period (Kubota et al., 1979; Jacquin et al., 1983a, b; Wilson et al., 1983; Walberg, 1984). Several factors may contribute to the actual number of labelled neurons observed in any experimental procedure. The mode of application of HRP, the site of injection; the status of HRP uptake and retrograde transport, survival period, fixation, histochemical reaction sensitivity and the possible loss of HRP reaction product during dehydration and mounting (Mesulam 1978, 1982). The sensitivity of HRP itself is very important. Sigma Type VI HRP has been found to be most sensitive for <sup>the</sup> retrograde transport method. In the early



stages of the present study, in some pilot experimental procedures in the rat, BDH-HRP yielded negative results.

4.45 Variability in the number of labelled neurons found following HRP application to tooth pulp may be as a result of inadequate transport from the site of injection (Arvidsson, 1975; Aker and Reith, 1981; Wilson et al., 1983). Aker and Reith (1981) suggest that there is no guarantee that all the terminal fibres of tooth pulp pick up and transport HRP. In their pilot experiments, they exposed tooth pulp and injected HRP. However, they found the pulp cavity to be extensively damaged and the findings extremely variable, with many negative results. With the technique of near-pulp exposure and HRP pellet rather than solution, the number of labelled neurons was less variable. Wilson et al. (1983), also found variability which may be due to cavity depth variation and the low concentration of HRP in some cases. Wilson et al., (1983), have also suggested that there may be leakage to the apical periodontium if large volumes of HRP solution are used.

4.46 On injecting the muscles of mastication, variation in the number of labelled mesencephalic and motor neurons has been found (Ibrahim and Leong, 1979; Walberg, 1984; Gottlieb et al., 1984). One of the reasons suggested for the variability may be that the HRP is not fully able to penetrate the capsule of the muscle spindle and thus not

all the receptors may take up HRP. More labelled cells are noted when HRP is applied to cut masseter nerve than when HRP is injected into the muscle. Similarly, in the periodontal ligament, more mesencephalic neurons were labelled following HRP application to inferior alveolar nerve rather than the periodontal ligament of mandibular teeth (Gottlieb et al., 1984). Moreover, when HRP was applied to sensory and motor branches of the trigeminal nerve, the total number of labelled cells for the whole nerve did not equal the sum for the individual branches (Jacquin et al., 1938a; Gottlieb et al., 1984).

4.47 In the present study, tracing neural connections of the periodontal ligament and gingivae comprises the major experimental work. Three methods of applying HRP were used. In the early experiments, 10 monkeys were available from the Institute of Primate Research. Tooth extraction was carried out and 30% HRP solution was injected into the socket. Different teeth types were extracted in these monkeys. The findings on HRP labelled neurons were negative for all the monkeys and this was attributed to either lack of experience of histochemical procedure or inadequate perfusion of the tissues or dilution and loss of HRP from the socket. Controlled procedures were then carried out in baboons<sup>No.</sup> 879, 880 and 881 which showed that<sup>HRP into</sup> intact periodontal ligament resulted in positive labelling in the trigeminal ganglion and mesencephalic nucleus as compared to negative results

when HRP was injected into <sup>the</sup> tooth socket. Tooth extraction may damage nerve endings (similar to pulp damage noted by Aker and Reith, 1981). There could also be dilution of the HRP due to bleeding and granulation tissue formation. Labelled mesencephalic and trigeminal ganglion neurons were observed in the monkey number 1, whereby the tooth socket following extraction was packed with cotton wool soaked in HRP after HRP injection. Thus, there may have been adequate HRP concentration in the socket to enable retrograde HRP transport along some of the nerve branches and terminals.

4.48 On injecting HRP into the intact periodontal ligament, especially in the anterior teeth labelled mesencephalic and trigeminal ganglion neurons were observed. It is however possible that the HRP may not have fully penetrated the capsule of some of the mechanoreceptors in the intact periodontal ligament. Also, the HRP concentrations in the apical region may not have been adequate due to the pressure of backflow during the injection. In some procedures, in addition to injection of HRP into the periodontal ligament, a small "window" was opened through the buccal mucosa and alveolar plate to the apex of the tooth and HRP injected around the apex. Due care was taken not to perforate the root into the pulp. The number of labelled neurons observed following HRP into intact periodontal ligament and apical window were comparable.

4.49 The periodontal mechanoreceptors are discrete and compound receptors which are thought to be encapsulated (Harris and Griffin, 1974 a, b) as well as the unencapsulated Ruffini type nerve endings associated with connective tissue fibres of the periodontal ligament (Lewinsky and Stewart, 1937 a, b; Fallin 1958; Everts et al., 1979; Byers 1985; Byers et al., 1986). It has been suggested that the capsule of the mechanoreceptors of periodontal ligament may slow down the uptake of HRP by the nerve endings and thus impair the retrograde transport to the cell body. The capsule of the muscle spindle in the muscles of mastication has also been thought to prevent the maximum concentration of HRP around the nerve endings (Gottlieb et al., 1984). However, HRP labelled mesencephalic and trigeminal ganglion neurons of the periodontal afferents have been observed in the present study and by Bosley et al. (1983), Gottlieb et al. (1984), following HRP injections into the periodontal ligaments of monkey, baboon and cat teeth. The receptors are thus able to uptake and transport HRP, which may be due to optimum HRP concentration around the capsulated and non-capsulated endings. Since Byers (1985); Byers et al. (1986), have shown that unencapsulated mechanoreceptors are present in the periodontal ligament, the HRP uptake may not be entirely dependant on the presence of the capsule.

4.50      The trigeminal ganglion neurons and the mesencephalic neurons show similarities of axonal transport characteristics and velocities (Byers and Mathews, 1981; Byers, 1984; Byers, 1985). The distribution of the mechanoreceptors and free nerve endings have been found mainly in the apical region of the tooth (Kubota and Osnai, 1977; Cash and Linden, 1981, 1982; Byers, 1985; Byers et al., 1986). Byers and Holland (1977) have shown the distribution of the trigeminal ganglion nerve endings in gingivae, junctional epithelium and periodontal ligament in rat molars. Byers (1985) further showed that the trigeminal ganglion neuron receptors were concentrated mainly in the apical region and were also found in the cervical region. The mesencephalic receptors were located in the apical region and comprised about 6-12% of the receptors in the cat periodontal ligament. The presence of mesencephalic mechanoreceptors in the alveolar periosteum, gingivae and palate has been disputed (Byers et al., 1986), although these mechanoreceptors have been thought to be present (Corbin and Harrison, 1940; Sakada 1974; Sakada and Okomoto, 1975; Linden, 1978). Thus the HRP injection into intact periodontal ligament, into the tooth socket and the "apical window" to the root apex would expose the receptors in the gingivae, periodontal ligament and alveolar periosteum to the injected HRP which would be

subsequently retrogradely transported to the mesencephalic and trigeminal ganglion neurons.

4.51 The negative findings in some experimental procedures may be due to the variability of the survival period, coupled with fixation procedure and the time interval between fixation and histochemical reaction (Ibrahim and Leong, 1979). In many of the baboons and monkeys obtained from the pool of animals used in the bilharzia study, perfusion was carried out through the common carotid artery. In some of the vervet monkeys, the isolation and cannulation of the carotid artery was hampered resulting in inadequate clearing of blood and poor fixation. When fixation was inadequate the heads of the monkeys and baboons were left overnight in the fixative at 4°C with the calvaria opened. The brainstem and trigeminal ganglia were dissected from the cranial cavity the following day and cleared of fixative with sucrose buffer at 4°C for another 12 - 24 hrs. The prolonged exposure of the fixative to the brainstem and ganglia may also have depressed the sensitivity of the retrogradely transported HRP in the neurons (Mesulam, 1982).

4.52 The procedure of histochemical demonstration of HRP granules may also explain the variability of findings. The histochemical method using TMB is the most sensitive for demonstrating the maximum number of labelled neurons

(Mesulam, 1978). There may be some variability depending on the optimum concentration of hydrogen peroxide and also due to loss of resultant reaction product of HRP granules during dehydration with alcohols. The visibility of blue HRP reaction product obtained with TMB reaction is best, when observed in brightfield microscopy within 48 hrs (Anderson et al., 1978). The observation of some lightly HRP filled neurons may have been difficult in some thick sections. All sections were observed to detect "labelled" neurons. Often, "brown" granules were observed. These may be the true HRP granules which change from blue to brown or it could be lipofuscin pigment which is occasionally present in neurons. When lipofuscin was present, it was generally found in variable amounts in most of the neurons of the ganglion and mesencephalic nucleus of the respective monkeys and baboons. The nature of the HRP reaction product may be fine granules or dispersed coarser granules. It is thought that the HRP reaction product is fine granular when transported by intact nerve terminals and coarser and dispersed when taken up by injured axons or perikarya. The HRP reaction product is thought to be more amorphous and finer when transported anterogradely (Mesulam, 1982). The distinction between "HRP labelled" neuron and some background "granular artifacts" was sometimes difficult and this may have resulted in under or over counting of labelled neurons.



4.53 An attempt was made in the present study to observe the electron microscopic appearance of the HRP labelled cells. It was not possible to show conclusively the electron microscopic appearance of the HRP labelled cells. This may be due to the problems of stability and sensitivity of HRP and some technical aspects of the HRP method which have already been discussed. Several experimental procedures and some modifications in the histochemical method may be necessary to demonstrate conclusively the HRP labelled cells at the electron microscope level (Itoh et al., 1979). The exogenous HRP is taken up at the axon terminal and upon retrograde transport, it ~~accumulates~~ in the lysosomes of the cell body and of large dendrites (Nauta et al., 1975). However, the HRP containing bodies can be positively identified with light microscope while with the electron microscope it can be difficult to distinguish HRP labelled neurons from unlabelled neurons containing lysosomes (Osculati et al., 1980).

4.54 Some monkeys and baboons were injected on both sides in different teeth types in the opposite jaw. Given similar experimental conditions, the quantification of the labelled neurons observed may reflect the relevant neural connections of the two teeth types injected in the same animal. Anterior teeth generally showed many labelled neurons compared to molars in the same animal as found in baboon 864 and monkey 4 (Table 5).

4.55 The highest numbers of labelled neurons were observed in well perfused tissues processed within 3 to 4 days following perfusion. The age of the animal appeared not to be critical as the number of labelled neurons in the young animals compared to the older ones did not differ much. The survival period of 48-72 hours for the monkeys and baboons was found to be adequate. In baboons 863 and 866 from the bilharzia study, survival period was 120 hours with two HRP injections on day 1 and day 4. However, the number of labelled neurons in baboon 863 exceeded those for 866 although experimental procedure and ages of the animal were comparable. Generally, fewer total HRP labelled neurons were observed in the baboon brainstem and trigeminal ganglia than in the monkey for similar teeth types. It is not clear whether the difference in the monkey and baboon is due to inadequate HRP concentration in the receptor site, different retrograde transport velocities, inadequate survival period in the baboon or any other reason.

4.56 The extent to which the number of labelled neurons observed for the various teeth types are a realistic reflection of their representation in the brainstem and trigeminal ganglia may depend on whether most of the nerve terminals have taken up the HRP and transported it retrogradely to the cell bodies which have been subsequently observed following TMB reaction. Possibly, the best cell counts, being the highest number of

labelled neurons for the various teeth types may give some indication of the quantitative neuron representation in the mesencephalic nucleus and trigeminal ganglion despite the limitations of the HRP method considered above.

C. Quantitative analysis of the mesencephalic nucleus of trigeminal nerve and the trigeminal ganglion and some unique features of the trigeminal system.

(i) Mesencephalic Nucleus of Trigeminal Nerve

4 57. The regional variations noted along the rostro-caudal extent of the monkey and baboon mesencephalic nucleus were similar to those seen in the cat and monkey (Weinberg, 1928; Sivanandasingham and Warwick, 1976; Capra et al., 1985). There are regions of neuron aggregation in the mid-collicular and rostral pontine region with sparcity in the caudal inferior collicular level. In the rat, the concentration of the cells have been noted in the pontine region (Weinberg, 1928; Rokx et al., 1986a). In the submammals, the cells are distributed in the dorso-ventral aspect of the optic tectum (Weinberg, 1928).

4 58. The standard deviation in the mean of cell counts for each 0.5mm of the brainstem as calculated for the various monkeys and baboons respectively, shows that there are variations in the density of cells along the

rostro-caudal extent from one animal to the next. The variations in the density of cells in each 0.5mm of the brainstems may be as a result of differences in the level of each 0.5mm of the brainstems along the rostro-caudal extent. The difference in the level may be due to the data having been obtained from sections of variable thickness from the individual brainstem. The observed differences in the cell counts of the right and left side in any one section may be due to the obliquity of the section, one side having been cut at a different level from the other. It may however be a real difference showing that the cell density is not similar on both sides at any one level.

4 59. Bilateral total cell counts were made from the serial paraffin wax sections of six monkeys and four baboon brainstems. The bilateral cell counts ranged from 810-1821 in the monkey with a mean of  $1341 \pm 380$  and in the baboon the range was 1312 to 2063 with a mean of  $1620 \pm 366$ .

4 60. There was a small difference of about 50-100 cells noted in the total cell counts of the right and left side of the mesencephalic nucleus in each brainstem showing that cell numbers were not equal on both sides as also noted by others in the rat and cat (Hinrichsen and Larramendi, 1969; Sivanandasingham and Warwick, 1976).

4 61. Unilateral total cell counts were made from the serial frozen sections (about 50 - 100 $\mu$ m) of the brainstems of 10 monkeys and 13 baboons from the HRP studies. The mean unilateral count was  $660 \pm 220$  in the monkey and  $797 \pm 304$  in the baboon.

4 62. Stereological analysis has been carried out on stratified serial paraffin-wax sections of brainstems of seven monkeys and four baboons. Volume density ( $V_V$ ), number per unit area ( $N_A$ ) and the numerical density, ( $N_V$ ) of the mesencephalic neurons in the brainstem was obtained. From the value of the numerical density and measured volume of the brainstem, the estimated total number of mesencephalic neurons has been found to be 2160 and 2674 in two monkeys and 2730, and 2816 in two baboons.

4 63. The present study shows variation in the values of total cell counts of the mesencephalic nucleus in the brainstems of various monkeys and baboons respectively. It appears that the total cell counts of the mesencephalic nucleus range from about 800 to 2600 in the monkey and 1300 to 2800 in the baboon. The differences in the values obtained from the cell counts of the mesencephalic nuclei of different monkeys and baboons respectively may be a real variation suggesting that the total number is not constant in any genus or within the species. The variation in the total number may however

be due to undercounting and overcounting of cells in the sections of varying thickness obtained from the brainstems of the monkeys and baboons. The cells may be undercounted in the sections where every 5th or 10th section is collected in the series of 10 $\mu$ m section thickness or overcounted in sections where every 5th section is collected in series of 7  $\mu$ m sections. In the cell counts from the frozen sections of 50 - 100 $\mu$ m thickness there may be an undercount of cells, <sup>as</sup> some of the neurons may not be easily visible in thick sections.

4 64. It is noted that the numerical density of the mesencephalic neurons obtained by two different formulae is not the same. The numerical density calculated from the formula which uses volume density (Weibel and Gomez 1962) is lower than one from the formula using mean diameter of neurons (Weibel, 1979). There are some errors involved in the counting of discrete structures in sections of finite thicknesses (Aherne, 1967; Weibel, 1979, 1980), and there may be errors involved in estimation of the diameter of neurons (Blinkov and Glezer, 1968). The measured volume of the brainstem prior to sectioning may be overestimated or underestimated. If the rostro-caudal extent of the brainstem exceeds the extent of the mesencephalic nucleus then there is an overestimate of volume. If the nucleus extends too far rostral or caudal to the anterior and posterior limit of the measured brainstem, then there is an underestimate.

There may also be a change in the volume of the brainstem due to perfusion fixation. There is also some shrinkage in the tissue during dehydration and embedding which has not been corrected for.

4 65. The errors involved in counting structures, the estimation of the diameter of the neurons and the section thickness have been discussed by several authors (Abercrombie, 1946; Konigsmark, 1970; Weibel, 1979). The method of stereology and morphometry have been expanded to use image analysis and computer analysis of the data (Aherne and Dunhill, 1982). The critique of the formulae used in the present study have been presented and recently other methods of determining estimates of the total counts of discrete structures have been proposed (Cruz-Orive, 1985; Gundersen, 1986).

4 66. The available data on the cell counts of the mesencephalic nucleus of the trigeminal nerve in the primates is variable and scanty. In one monkey Kosaka (1912) found 2744 cells, Weinberg (1928) found 4869 cells while Sivanandasingham and Warwick (1976) found 862, 968 cells (unilateral) in two rhesus monkeys and 467, 498 cells in two slow loris. The cell counts in an adult human brainstem has been found to be about 5735 (Weinberg, 1928), and in 5 month old infant to be 741 (Valkenberg, 1909).



4 67. The data on the total mesencephalic neurons in the cat and rat are also variable from different studies (Weinberg, 1928; Sivanandasingham and Warwick, 1976; Walberg 1984; Capra et al., 1985; Rokx et al., 1986a). Most recent studies show that the total cell counts of the mesencephalic nucleus is about 900-1000 (unilateral) in the cat (Gottlieb et al., 1984; Walberg, 1984; Capra et al., 1985). In the rat (Sivanandasingham and Warwick, 1976), counted 638-748 cells while Rokx et al. (1986a), found 1000-1600 cells. Both the studies used the criteria and method for cell counting proposed by Konigsmark (1970). The value of total cell count of 578 in the rat agrees with that of Foster (1973) while that of 1600 agrees with that found by Rakhway et al. (1972), while Hinrichsen and Larramendi (1969) found about 2434 cells in the rat.

4 68. Thus there is a variation in the number of cell counts obtained by different methods as well as similar methods in the same species and also an apparent variation from one animal to the next of the same species. The differences in the mean total cell count between the monkey and baboon is significant ( $P < 0.1$ ). The larger number in the baboon may be a reflection of difference in body size between the monkey and baboon. However, body size relation is not clear since the values for rat and cat obtained from some studies are comparable to that found in the monkey and baboon. Taking the low

count for rat 500 cells, cat 900-1000 cells, vervet monkey 1300 cells and the baboon about 1800 cells, it appears that the difference in cell numbers may be as a result of body size difference, species and genus difference. Marini and Bortolami (1982) found that unilaterally in the water frog weighing 50gm, there were 180 mesencephalic neurons while in the bull frog weighing 350 gm there were 250 neurons.

4 69. The mean cell diameter of the monkey mesencephalic neuron has been found to be  $33 \pm 6\mu\text{m}$  and of the baboon to be  $39 \pm 8\mu\text{m}$  (corrected value  $38\mu\text{m}$  and  $45 \mu\text{m}$  respectively); The difference in size of neuron is significant ( $p < 0.1$ ). The larger cell size in the baboon may possibly account for the larger area of innervation as compared to the small head of the monkey. The size of cell in the monkey compares well with that in the cat and rat (Walberg 1984; Capra et al., 1985; Nomura et al., 1985; Hinrichsen and Larramendi, 1969; and Rokx et al., 1986a). The variability in cell size and morphology of the different neurons of mesencephalic nucleus in the monkey and baboon is also comparable to that observed by Weinberg (1928), Sheinin (1930) and Capra et al. (1985), in the rat, cat and dog. Capra et al. (1985), found the mean cell diameter of the mesencephalic neurons of the cat to be  $35\mu\text{m}$  with a range of  $17-73\mu\text{m}$  using method of image analysis of HRP labelled cells. Nomura et al. (1985), found the maximum diameter to be  $28.2 \pm 0.42\mu\text{m}$

for the large pseudounipolar cells and  $24.5 \pm 0.57 \mu\text{m}$  for the smaller multipolar cells in the cat on analysis of HRP labelled cells.

4.70. The data obtained from the present study shows that the difference in the mean total number of mesencephalic neurons in the monkey and baboon is significant and the size of the neuron in the baboon is significantly larger than that of the monkey. It is plausible that the arborization of peripheral fibres of the larger and more neurons of the baboon mesencephalic nucleus innervates a larger area in the bigger animal as compared to the smaller monkey.

(ii) The Trigeminal Ganglion

4.71 The volume density ( $V_V$ ) number per unit area ( $N_A$ ) and numerical density ( $N_V$ ) of the trigeminal ganglion neurons of the vervet monkey and olive baboon were estimated using stereological methods. From the numerical density and the measured volume of the ganglia, total number of neurons in the ganglion has been estimated. The mean estimated number in the monkey ganglion has been found to be  $101178 \pm 6903$  and in the baboon to be  $137250 \pm 10274$ . In the young baboon,  $N_V$  is about 2000 neurons per  $\text{mm}^3$  and in the adult baboon,  $N_V$  is about 1000 per  $\text{mm}^3$ . The estimated total number of ganglion neurons has been found to be between about 120,000 and 140,000 in both, the young and adult baboon.

4.72 The estimate of the total cell counts of the trigeminal ganglia of the vervet monkey and olive baboon obtained by stereological method may be an underestimate due to the correction of the measured volume for the non-ganglionic tissue. It was difficult to ascertain exactly the region of the ganglionic band, thus gross trimming of the ganglion included areas of non-neuronal regions and hence correction was made in the measured volume with respect to the "ganglionic reference area". There may also be errors involved in counting discrete neurons with a clear nucleus and nucleolus. This would influence the value of  $N_A$ , number per unit area. The estimation of the mean neuron diameter may also provide a source of error since the size distribution plots for the neuron profiles were not done. In addition, there could also be errors from tissue shrinkage, swelling and dehydration and section thickness (Abercrombie, 1946).

4.73 The total cell counts of the trigeminal ganglion neurons were also obtained by counting the number of neurons per section and multiplying the mean number per section by the total number of sections obtained from a ganglion. The mean total cell count in the monkey ganglion has been found to be  $98,073 \pm 7613$  and of the baboon to be  $153,555 \pm 11343$ . Errors in counting the number of neurons in the section and number of sections of the ganglion would provide a source of error in the total number of neurons, although this is one of the

methods which has been suggested for obtaining total neuron counts (Blinkov and Glezer, 1968). The values of total cell counts obtained by stereological methods were close to those obtained from counts per section. The difference between the mean of total cell counts of the monkey and baboon ganglia is significant ( $P < 0.01$ ).

4.74        Thus it appears that the trigeminal ganglion neurons innervating the head region in the monkey are about 100,000 in the baboon about 140,000. In the monkey, the number of neurons may be less than that in the baboon due to the smaller body size of the monkey. However, in the young baboon aged about 2 yrs age, the number of neurons is similar to that in the older baboon, although the numerical density is almost twice that in the older baboon. There is an almost two fold increase in the volume of the trigeminal ganglion from the young to the old. It is likely that the volume of the axons and blood vessels may increase rather than the actual number of neurons. A small increase (about  $5\mu\text{m}$ ) in the size of neurons between the young and adult baboon has been observed. In the humans, an increase in the number of spinal ganglion neurons upto 3 years has been noted (Lieberman, 1976).

4.75        The corrected cell diameter of the monkey ganglion neuron has been found to be about  $53 \pm 12 \mu\text{m}$  and of the baboon neuron to be about  $60 \pm 13 \mu\text{m}$ . The difference

between the monkey and baboon neuron is significant ( $P < 0.001$ ). The large cell size in the baboon may be due to body size difference as well as the larger peripheral area of innervation of the neurons. In the young baboon, the number has probably reached maximum. There is some increase in cell size of the ganglion from the young to the adult which together with the increase in size of the axon and peripheral arborization may cater for the increase in the size of the area of innervation<sup>of</sup> the head, with growth of the animal.

4.76 There is a positive correlation between cell size and axon diameter (Ramon y Cajal, 1909). There is also a positive relationship between ganglion cell size and body size, the largest neurons occurring in the ganglia of large mammals (Lieberman, 1976). In addition there is positive relationship between cell-body size and size of peripheral area innervated. An increase in the size of cell-body is noted with normal postnatal growth (Pannese, 1963 and Lieberman, 1976). In man, increase in size of the cell body of the spinal ganglion cells is noted till 12 years. (Otha et al., 1974)

4.77 There appears to be no known data on the cell counts of the trigeminal ganglia of non-human primates (Blinkov and Glezer, 1968; Hill, 1976). The total neuron counts of the ganglion in man is not known though 140,000 sensory fibres have been estimated in the sensory root of

the ganglion (Sjoqvist, 1938). According to Belyaev (1963) the number of motor root fibres varies from 6348 to 14601 and sensory root from 76, 842 to 150,079 in man. Assuming that each sensory root fibre accounts for the respective neuron in the ganglion, the total number of ganglion neurons in the monkey and baboon compares well with that in man. The trigeminal nerve in the baboon has many similarities to that in man, although the maxillary and mandibular divisions are large due to the marked prognathism in the baboon (Gasser and Wise, 1972). The vervet monkey is comparatively small and thus the less number of neurons, compared to man. The comparative total cell counts in the trigeminal ganglia of the monkey, baboon, and man may also be related to functional similarities of the structures innervated by the ganglion neurons.

4.78 The number of cells per ganglion shows variation in the monkey and in the baboon. There is also some difference in the number of neurons in the right and left ganglia. The total number of trigeminal ganglion<sup>neurons</sup> in the rat has been found to be variable, with a wide range. Gregg and Dixon (1973) counted between 40,910 and 62,030 cells with a mean of 49350 in 12 ganglia. They counted neurons in every 10th section of the 10 $\mu$ m thickness. Aldoskogius and Arvidsson (1978) found 23279-46713 number of ganglion neurons in 4 rats, counting neurons with nuclei in every 5th section of 15  $\mu$ m thickness. Thus,



there is some variation in the total ganglion cells of the same species and between species. The differences in the total number of trigeminal ganglion neurons in the rat, monkey and baboon may most likely be due to body size differences.

4.79 The variation in the number of neurons in sensory ganglia at cervical, thoracic and lumbar level is also notable in the available data on total cell counts of spinal ganglia in man and other animals, (Blinkov and Glezer, 1968; Leiberman, 1976). The cell counts in the thoracic spinal ganglia in man ranged from 24000 to 36000, and in the cat 7000-14000. The variation in number of the spinal root ganglion along the cervical, thoracic and lumbosacral region has also been noted in the same animal and this is attributed to the differences in the area of distribution.

4.80 Using two methods, stereological analysis and cell counting, data has been obtained in the present study showing some variations in the total cell counts in the mesencephalic nucleus and trigeminal ganglia of the various monkeys and baboons. Apart from the differences in the methods of counting neurons, and the histological procedures that may cause diversity of total cell counts within the species noted in the available data in the cat, rat, monkey and baboon, (Abercrombie, 1946; Konigsmark 1970; Sivanandasingham and Warwick, 1976; Rokx

et al., 1986a); there may be other factors that account for the variation in total number of neurons in the mesencephalic nucleus and trigeminal ganglion in the same species. The age of the individual, the nutritional as well as metabolic and environmental differences may account for the variability (Weinberg, 1928; Gregg and Dixon, 1973). There may be loss of neurons from many parts of the brain (Dayan, 1971). Both the size of peripheral innervation area and environmental influences during certain periods of development may have an effect on the number and morphology of cells (Cavanaugh, 1951).

4.81 Functionally, the mesencephalic neurons of trigeminal nerve subserve proprioceptive modalities from the oro-facial region. The peripheral process exits with the motor root and sensory root of trigeminal nerve and are distributed to the muscles of mastication, periodontal ligaments of teeth and to the mucosa of the palate and gingivae (Weinberg, 1928; Linden, 1978; Ryu and Kawana, 1984; Gottlieb et al., 1984; Capra et al., 1984; 1985). About 70% of the mesencephalic neurons innervate the stretch receptors of the jaw elevator muscles while about 20-30% innervate the periodontal ligament and gingivae of teeth (Corbin, 1940; Gottlieb et al., 1984; Nomura et al., 1985). About 10-15% of mesencephalic neurons were found to innervate the incisors, canines and molars ( $M^1 + M^2$ ) in the present study. The distribution of mesencephalic afferent fibres

to the extraocular muscles of the eye is still disputed (Corbin and Harrison, 1940; Fillenz, 1955; Gabrawi and Tarkhan, 1967; Cody et al., 1972, Sherif et al., 1981; Byers et al., 1986).

4.82 The trigeminal ganglion neurons innervate skin, oral mucosa, nasal mucosa, cornea and periodontal ligament and tooth pulp (Miles 1979). The trigeminal ganglion neurons are also thought to innervate the proprioceptors of the extra ocular muscles in the cat and monkey (Porter and Spencer, 1982; Porter et al., 1983.). The capsule of the temporomandibular joint in the cat is shown to be innervated by the ganglion neurons (Romfh et al., 1979). The afferent cell bodies of general sensory receptors and proprioceptors of the tongue mucosa and muscles in the monkey are thought to be present in the first and second cervical spinal ganglia (Fitzgerald and Sachithanandan, 1979).

4.83 The numbers of labelled trigeminal ganglion neurons were more than the numbers of mesencephalic neurons for the periodontal ligament and gingivae of incisors, canines and molars both in the monkey and baboon. This may be because there are more receptors of general sensations in the gingivae and the periodontal ligament having the cell bodies in the trigeminal ganglion. The proprioceptive mechanoreceptors with the cell bodies in the mesencephalic nucleus may be fewer in number.

However, in the monkey, the labelled neurons of the incisors, canines and molars represent 0.27% 0.22% and 0.05% respectively of the total number of trigeminal ganglion neurons. The labelled mesencephalic neurons in the monkey of the incisors, canines and molars represent about 6%, 4.4% and 4.5% respectively of the total number of mesencephalic neurons.

4.84 The values for the volume percent of the labelled ganglion neurons for incisors, canines and molars obtained by stereological analysis was 1.7% for incisors and canines and 0.25% for molars in the monkey. Errors may be involved in the estimate of percentage of labelled neurons from actual counts as well as <sup>the</sup> stereological method. The former may be an under estimate since some faintly labelled neurons may have been missed in counting labelled cells in frozen sections of 50-100  $\mu$ m thickness. The latter may be an overestimate as the analysis of sections by point counting may be biased towards sections with heavy labelling giving an over all higher estimate of volume density of labelled neurons.

4.85 Nevertheless, it appears that there are fewer specialized neurons from a small pool of neurons subserving the special function of proprioception while there are more neurons from a relatively large pool of neurons subserving general sensations of pain , pressure, temperature and touch.

4.86 The mesencephalic neurons are unique in being the primary cell bodies located within the central nervous system. The morphological features of the mesencephalic neurons are generally similar to those of the trigeminal ganglion neurons, but there are some unusual differences between the two cells, (Lieberman 1976). The cell population of the mesencephalic nucleus in the monkey, baboon and other animals is heterogeneous, consisting of mainly large and medium size spherical and oval unipolar cells as well as some bipolar and multipolar cells (Weinberg, 1928; Shenin, 1930; Walberg, 1984; Capra et al., 1985). The trigeminal ganglion neurons in the monkey and baboon are large and small sized cells with the characteristic morphological cell features of cranial sensory ganglia (Lieberman 1976).

4.87 Soma-soma contacts between some of the mesencephalic cells in cluster in the monkey and baboon have been observed at the electron microscopic level. Axosomatic and soma-somatic contacts of the mesencephalic neurons have also been observed in the cat, rat and other animals (Ramon y Cajal, 1909; Hinrichsen and Larramendi, 1970; Walberg, 1984). Synaptic contacts have also been described in the mesencephalic neurons (Hinrichsen and Larramendi, 1970; Nomura et al., 1985). Synaptic contacts have not been observed in trigeminal ganglion neurons (Lieberman 1976).

4.88 Some developmental aspects of the mesencephalic nucleus are also notable. The neurons may develop from dual origin, neural crest and the ectodermal plate. (Kosaka, 1912; Dubner et al., 1978). Developmentally, mesencephalic neurons appear very early. In the baboon, the mesencephalic neurons are fully differentiated by <sup>day</sup> 70 (Hassanali et al., 1984). In the rat 80% of the neurons are differentiated by day 11 (Narayan and Narayan, 1985). Mesencephalic tract is one of the earliest myelinated structures of the brainstem which apparently increases in size according to the number of fibres amongst the primates (Gerhard and Olszewski, 1969). Whether the variations in some of the histological descriptions of the mesencephalic neurons in different species and in the young and older animals may reflect functional, phylogenetic or ontogenic differences is not clear (Dubner et al., 1978). The dorsal and dorso-ventral position of the mesencephalic neurons has been thought to be related phylogenetically (DuBrhul, 1960). The caudal location of the mesencephalic neurons innervating the teeth may so that these neurons approximate closer to the target organs. The ventral position of the neurons in mammals is thought to be due to migration of the neurons to be nearer to head muscles (Corbin and Harrison, 1940).

4.89 The size of the trigeminal ganglion neurons has been found to be larger (53  $\mu$ m in monkey and 60  $\mu$ m in baboon) than the mesencephalic neuron (38  $\mu$ m in the

monkey and 45 um in the baboon) in both the monkey and baboon. Similar observations have been made in the cat (Jones et al., 1982 Capra et al., 1985). The neuronal processes are assumed to be proportional to the size of the perikarya (Corbin, 1940; Lieberman, 1976). The fibres of the mesencephalic neurons may be limited in size (Luschei and Goldberg, 1982) projecting to very specific sites such as some muscles and periodontal ligaments of teeth and are concerned with the specific function of proprioception (Corbin and Harrison, 1940; Byers et al., 1986). The large axons near the cell body becomes smaller towards the periphery and arborization of the cell process has been noted (Byers et al. 1986). Some of the single cells may thus innervate more than one tooth (Linden, 1978; Byers et al., 1986).

4.90 The trigeminal ganglion neurons **have** greater diversity of fibre spectrum and projects to large areas of the head and subserves the modalities of general sensations of pain, pressure, temperature and touch (Pannese, 1963; Lieberman, 1976). The trigeminal ganglion neurons innervate the oral mucosa, periodontal ligament as well as the dental pulp. The peripheral fibres of the trigeminal ganglion neurons also arborise, the same neurons having a large receptive field (Darian-Smith, 1973). However, the ganglion neuron which innervates the periodontal ligament does not seem to send a branching collateral to the pulp (Capra et al., 1984). The intra and perioral area may be innervated by larger cells and periphery of <sup>the</sup> head by smaller cells (Sugimoto et al. 1986).



4.91 Some aspects of the trigeminal innervation of the head region are unique (Dubner et al., 1978; Miles, 1979; Kruger and Young, 1981). The proportion of the myelinated to unmyelinated fibres are higher in the trigeminal nerve than spinal nerves (Blinkov and Glezer, 1968, Young and King, 1973). The tooth pulp has a high rate of myelinated A delta fibres compared to the unmyelinated C fibres (Kubota et al., 1982). The organization of the trigeminal brainstem nuclear complex is also unique (Sessle and Greenwood, 1976 a, b). The various nuclei are somatotopically organized (Kruger and Young, 1981). There are internuclear connections and interneurons that modify the thalamic projections and are involved in reflex actions in mastication and swallowing. (Sessle, 1976). Compared to the spinal cord, the afferents of the brain are 130% greater than the spinal cord. Of all the 1,300,000 cranial afferents in man, the trigeminal afferents account for 140,000 after the optic nerve which has 1,000,000. In the spinal nerves, for each motor fibre, there are five sensory afferents while for the cranial nerves, in the trigeminal, there are 19 sensory afferents for each motor nerve (Blinkov and Glezer, 1968). Sensory afferents and peripheral feed back from the head region to the central nervous system appears to be a very important aspect in co-ordination and integration within the central nervous system, particularly in the head region.

4.92 The innervation of teeth is an unusual feature of the trigeminal system. The teeth are specialized structures in which the pulp is an innervated soft tissue surrounded by hard tissue. The conduction of sensation from pulp/dentine is still poorly understood. The sensation of pain and perhaps touch is elicited from the pulp. It may be that suprathreshold stimuli produce only pain and other sensations are masked by pain evoked from the pulp (Dubner et al., 1978). The periodontal ligament or the periodontium provides a major source of peripheral sensory information about the position of the tooth in space, i.e. its relationship to other teeth, mouth structures as well as food in the mouth. Some of the afferents from the periodontium travel with muscle and joint afferents and are part of the proprioceptive system in the oro-facial region. These proprioceptive teeth afferents have a particular significance since they are activated during tooth contacts and thus can influence simple as well as complex reflex activity (Dubner et al., 1978, Van Steenberge 1979).

D) Functional Considerations of the Neural Connections of the Teeth.

4.93 The neurophysiological and functional aspects of the afferent connections of the teeth to the trigeminal ganglion and mesencephalic nucleus involves mostly the role of the teeth in the normal function of mastication

(Kawamura, 1974., Dellow, 1976; Hartman et al., 1979; Luschei and Goldberg, 1982.) During mastication, the movement of the mandible is such that the dental arches are aligned to transmit biting forces from the teeth to the food mass that is being chewed (Ahlgren, 1976). Mastication thus involves a complex series of movements that differ depending on the amount of chewing necessary to form the bolus of food of any particular consistency (Ahlgren and Owall, 1979, Hiimae, 1978). Occlusal forces applied to the teeth during mastication stimulate the sensory receptors in the gingivae and the periodontal ligament (Anderson et al., 1970; Hannam, 1976; Dubner et al., 1978).

4.94 The trigeminal ganglion neurons are thought to subserve general sensations while periodontal mesencephalic neurons subserve proprioceptive sensation (Hannam, 1976). The central processes of the trigeminal ganglion neurons synapse with the main sensory and spinal nucleus of the trigeminal nerve and may project to the sensory cortex along the thalamocortical pathway or be relayed at segmental levels (Hannam, 1976). Connections with the alpha motor neurons of muscles of mastication are established through interneurons located in the supratrigeminal nucleus and sensory nucleus (Jerge, 1963b, 1964; Kidokoro et al., 1968; Sumino, 1976; Dubner et al., 1978). The periodontal afferent neurons which have their cell bodies in the mesencephalic nucleus are

also involved with reflex activity in the muscles of mastication. There are collateral projections from the mesencephalic neurons to the motor nucleus of trigeminal nerve, many of these mesencephalic projections being concerned with relaying afferent information from the muscle spindles (Hannam, 1976). The periodontal mesencephalic connections to the motoneurons may be through the interneurons located in the supratrigeminal nucleus (Jerge, 1964). It is possible that the interneurons of the supratrigeminal nucleus may be involved in the bilateral control of muscle behaviour during mastication (Dubner et al., 1978; Rokx et al., 1986b). Periodontal mesencephalic afferent neuron projections to the cerebellum have also been observed suggesting a cerebellar modulation of muscle activity (Cody and Richardson, 1979; Elias et al., 1985).

4.95 The neural control of mastication is due to an integration of cortical, brainstem and peripheral modulation (Dubner et al., 1978). Sherrington (1917), elicited reflex jaw opening on intra-oral stimulation of teeth. Intra-oral stimulation of the periodontal ligament and oral mucosa causes excitatory post-synaptic potentials in the diaphragmatic motoneurons and reflex inhibition of the motor neurons innervating jaw-elevator muscles (Bratzlavsky, 1976; Goldberg, 1976; Erkelens and Bosman 1985; Yamada et al., 1985). The jaw-opening reflex can be elicited by stimulation of intra-oral

nociceptors and periodontal mechanoreceptors (Hannam and Matthews, 1968, 1969; Yemm, 1972 ). The response to nociceptors may be a protective function similar to the spinal withdrawal reflex. The stimulation of mechanoreceptors may be more complex in eliciting reflexes associated with chewing function (Dubner et al., 1978). Stimulation of periodontal receptors may also cause the silent period observed in the jaw elevator muscles following tooth contact (Hannam et al., 1969, 1970; Owall and Elmquist, 1975). The jaw-jerk occurs as a result of the myostatic monosynaptic stretch reflex of jaw elevator muscles (Szentagothai, 1948; Bratzlavsky, 1976). Although reflex mechanisms are considered to be important during mastication, and may provide some information on the efferent and afferent connections of the structures involved, many normal and experimental phenomena related to mastication cannot be explained purely on the basis of reflexes (Lund, 1976). Firstly, jaw reflexes are of very short duration (100 msec) while jaw movements during normal mastication are repeated about once every second (Hedegard et al., 1970). Secondly, destruction of the mesencephalic nucleus in the monkey results in the loss of the refined mandibular movements but the rhythmic jaw movements during mastication remain unaffected (Goodwin and Lushei, 1974). Local anaesthesia of the teeth and the temporomandibular joint causes no significant alteration

in masticatory rhythmic chewing movement in human subjects (Schaerer et al., 1966). Matthews et al. (1969) found that the reflex inhibition of masseter neurons is not abolished by local anaesthesia of the tooth. However, the ability to position the food between the teeth was lost due to anaesthesia (Schaerer et al., 1966) and impaired in monkeys following mesencephalic lesions (Goodwin and Luschei, 1974). The biting force in the human subjects was altered due to local anaesthesia of the periodontal ligament (Orchardson and Macfarlane, 1980). Thus peripheral feedback from teeth may play a significant role in influencing and modifying the masticatory pattern.

4.96 Afferent stimulation from the intra oral receptors during chewing results in active digastric response and inhibition of elevator motor neurons (Sumino, 1976). However, the interneurons and pre-synaptic mechanism has to be involved during mastication in such a way that inhibitory afferents from oral mechanoreceptors to jaw closing motor neurons are shut down <sup>in the</sup> same way as excitatory pathways to the digastrics. This allows vigorous elevator activity to occur in the face of intra-oral afferents (Goldberg, 1971, 1972). Thus, instead of opposing jaw-closure during chewing, intra-oral sensory neurons could provide positive feedback resulting in controlled muscle activity (Appenteng et al., 1982). It has been shown by Lund and

Lammarie, (1973) that biting forces generated by jaw-closing muscles fall when periodontal receptors are blocked by local anaesthesia.

4.97 The trigeminal ganglion neurons innervating the periodontal ligament and tooth pulp would most likely be involved in consciously perceiving the sensations of touch, light pressures and pain and also be involved in signalling the position of the food bolus and perceiving size and consistency of food within the mouth. The mechanoreceptors which have a proprioceptive function are probably concerned more with the reflex jaw opening during occlusal contact (Dubner et al., 1978). Upon tooth contact, closure of the jaw is stopped by an increase in afferent impulses from the periodontal ligament to the muscles of mastication to inhibit further closure (Ahlgren, 1969; Andersen, 1976). Since jaw-opening muscles have few spindles, the periodontal receptors serve as a source of feed back for jaw-opening reflex (Rakhway et al., 1971; Gill, 1971; Dymtruk, 1974; Karlsson, 1976).

4.98 The ascending network of neurons which is associated with relaying tactile information from the teeth has prompted the suggestion that functionally, the periodontal ligament has a dual innervation; one set of receptors being involved in the mediation of brainstem reflexes, and the other with the conscious perception of



forces on the teeth (Jerge, 1967). However, Hannam (1976) does not support this suggestion. While it is true that brainstem reflexes can be evoked by the stimulation of the periodontal mechanoreceptors and that some receptors are involved with sensory stimulation there is no proof as yet that two functionally different sets of neurons exist. Moreover, recent evidence demonstrates that the periodontal innervation may have a positive feedback upon jaw closing motoneurons at the cortical level, in addition to its influence in the brainstem (Lund and Lamarre, 1973; Lund and Sessle, 1974). When other integrative actions of periodontal input are considered for example, the interaction at brainstem level with laryngeal inputs (Sessle, 1973) and at the cortical level with muscle afferents (Lund and Sessle, 1974;), it is apparent that the periodontal innervation has<sup>a</sup> more complex role to play than that of a simple innovator of brainstem reflexes in the jaw muscles and a transducer for a conscious sensory experience.

4.99 The observations in the present study show a possible bilateral projection from the dental afferents to the presumable supratrigeminal nucleus. The role of interneurons in modulating bilateral oral motor control as well as modulating the inhibitory and excitatory disynaptic reflex pathways to jaw-closing and jaw-opening motor neurons has been proposed (Dubner et al., 1978; Sumino, 1976). It is plausible that the mechanoreceptors

with the cell bodies in the mesencephalic nucleus respond to larger forces conducted by large myelinated nerve fibres and cause the early reflex inhibition of the masseteric motor neurons possibly through interneurons in the (supratrigeminal nucleus. The mechanoreceptors and nociceptors of the periodontal ligament and gingivae with afferent cell body in the trigeminal ganglion may be involved in the later inhibition of the masseteric motor neurons and excitation of the jaw opening motor neurons through the interneurons in the sensory trigeminal nucleus and supratrigeminal nucleus (Jerge, 1964; Sumino, 1976; Dubner et al., 1978).

4.100 Apart from the peripheral reflex modulation of masticatory behaviour, cortical influence on masticatory cycle has been proposed by Rioch (1934). An alternative concept is that there is "masticatory rhythm pattern generator" (chewing centre) probably located in the pontine reticular formation, which is capable of elaborating the basic cyclic pattern of muscle activity expressed in mastication (Magoun et al., 1933; Dellow and Lund, 1971; Sumi, 1971). The nature of the chewing centre is not clear but it may be composed of interneurons associated with motor neurons. The chewing centre can be activated from higher centres of the brain as well as by stimulation of the mouth by a bolus of food (Dubner et al., 1978). The sensory input from the oral cavity may ensure maintenance of the rhythm and modify it

when necessary. However, Luschei and Goodwin (1974, 1975) and Matthews (1975) have expressed doubt as to whether the so-called "chewing centre" can, by itself elaborate normal masticatory function per se which appears to be dependent on peripheral sensory feed-back.

4.101 The connective tissue and neural elements of the periodontal ligament are organized for support and attachment of the root (Shuttleworth and Smalley, 1983) as well as for sensory function (Stella, 1975). Force and direction are important parameters in determining whether a reflex response is likely to occur with mechanical stimulation of a tooth or not. It has been shown that teeth make contact during chewing (Haddad et al., 1974). The tooth contacts in masticatory movements are unique as the movements terminate with contact between two hard surfaces, which is not found to occur in any other functional situation in the body. Another unique feature is the restriction imposed on the final stages of closure movement by the shape of the tooth cusps (Anderson, 1976). The frequency of tooth contact during a chewing cycle increases towards the end of the cycle, but with some food materials, contact may occur with nearly every chewing stroke (Ahlgren, 1966; Anderson and Picton, 1957; Graf and Zander, 1963; Moller, 1966). Tooth contact is not a static position, but rather during contact, the lower jaw moves along a pathway determined by the shape of the tooth cusps (Adam and Zander, 1964).

During the occlusal phase of chewing, the force exerted by the elevator muscles is distributed over a greater tooth surface area than at any other stage of chewing cycle. Although the periodontal ligament cushions the shock resulting from the tooth contact, the maximum cushioning effect is probably no more than 0.05 mm (Picton, 1963). Studies on forces generated in chewing are limited (Carlsson, 1974; Haddad, 1976). Graf et al. (1974), and Graf (1975) measured forces in human teeth using the sound transmission method. The receptor thresholds for axially directed forces are higher than those for the tangentially directed forces. Graf (1975) found that balancing side forces are lower than working side forces which agrees with EMG studies of muscles (Moller 1966). Studies on oral tactility during chewing in the natural dentition (Owall, 1974) and oral tactility and sensibility during biting and chewing (Owall and Moller, 1974) in natural and artificial dentition have shown the role of periodontal ligament receptors during mastication.

4.102 It is generally agreed that chewing is a function which involves tooth contacts in the lateral gliding phases as well as in intercuspal position (Anderson, 1976; Dubner et al., 1978). In chewing, the greatest duration and magnitude of forces occur at the intercuspal position. It has been shown that teeth do not make contact on every masticatory stroke and about 20 to 84%

of masticatory strokes make tooth contact (Ahlgr n 1966, Pameijer, et al., 1968, 1969; Graf and Zander 1963; Schaerer and Stallard 1966). During the occlusal phase, as the lateral and forward gliding in contact begins, the receptors sensitive to horizontal movement will fire due to the directional sensitivity which is a property of the periodontal mechanoreceptors. The dramatic change in periodontal input comes from those teeth which were not load bearing during the initial stages of movement, but make contact in the terminal phase of the movement. Thus, sudden increase in total mechanoreceptor discharge distinguishes contact from the early stages of closure. Anderson (1976) has discussed the frequency of tooth contact in normal chewing and the role of mechanoreceptor discharge of the non-load bearing teeth in control of masticatory movements. A proper understanding of the role of teeth in mastication is made difficult as mastication is not an entirely reflex activity, but is accessible to conscious control which is very likely to be an important factor in experimental conditions (Dubner et al., 1978).

4.103 It is conceivable that the caudally located mesencephalic periodontal afferent neurons with the proximity to the motor nucleus are involved primarily in the modulation of the complex mandibular movements during the occlusal phase of chewing. In primates particularly where mandibular movements are lateral as well as

vertical compared to the cat where the movements are mainly vertical, these reflex jaw movements during the occlusal phase may be significant in producing shearing controlled forces between the teeth and the input from anterior as well as posterior teeth could be essential. The trigeminal ganglion afferent neurons may be concerned with the conscious perception of tactile forces on teeth and gingivae, reflexly positioning the food bolus in the mouth as well as reflex jaw-opening in later stages of masticatory cycle.

4.104 The origin and evolution of the masticatory apparatus and the influence of the mouth on the evolution of the brain has been considered (Young, 1968; DuBruhl, 1974; Poole, 1976). The developmental aspects of the masticatory cycle appears to depend on periodontal stimuli from teeth (Dubner et al., 1978). Ontogenetically, it is still not clear whether the post-natal expression of mastication in <sup>the</sup> human utilizes the neuromuscular mechanisms already existing for the function of suckling or whether new neural mechanisms may be developed or triggered by the eruption of teeth (Dubner et al., 1978). Although most of the structural requirements for mastication are present at birth in the mammals, including man, mastication is not manifested as a behaviour or function until well after birth. The absence of teeth during neo-natal period seems to be the main factor for the lack of mastication <sup>(Bosma, 1972, 1973)</sup>. Suckling is the

first form of feeding mechanism in the infant. There is evidence in the human foetus that reflex jaw opening can be obtained late in the first trimester and that by the beginning of the third trimester period, stimulation of the mouth can elicit what appears to be suckling movements (Humphrey,<sup>1970,</sup> 1972). Thus, at birth, the required neuromuscular mechanisms are operative for suckling. From the available evidence, it seems that learning has a role to play in suckling and that stimuli which are ineffective in modifying suckling in the neonate become powerful modifiers in the post-natal period (Papousek 1967; Sameroff, 1973). Suggestions have been made that mastication per se is expressed on the framework provided by the skeletal, joint and neuromuscular components operative in suckling (Dellow, 1969; Sessle, 1976).

4.105 Alternatively, it has been argued that mastication does not develop from suckling but is associated with completely new neuromuscular developments that may be triggered by the eruption of teeth (Bosma, 1967; Moyers, 1973). EMG recordings from masticatory muscles at various ages in the young have shown that EMG muscle pattern exhibit adaptations linked to various stages of development of teeth (Moyers, 1964). The learning process in mastication is also indicated by the fact that the masticatory movements in the child in early stages are uncoordinated but gradually become smooth and efficient for mastication of food. Ramfjord and Ash



(1971) state that with the growth of the infant and the eruption of the teeth, afferent stimuli from the receptors in the periodontal ligament influence the central nervous system and reflexely influence the position of the mandible. With eruption of teeth, the process of mastication is learned, and learning also depends upon the cerebral cortex (Dubner et al., 1978).

4.106 The available evidence does not allow for a definitive picture on the development of mastication. It has been postulated, that the cyclic jaw movements seen in suckling may form the basis of rhythmic or cyclic movements of the jaw during mastication, generated by the chewing centre in the brainstem (Dubner et al., 1978). At a critical time that may be related to the eruption of teeth, sensory information from the teeth and oral cavity may be super-imposed on the "chewing centre" in the brainstem modifying the jaw movements associated with mastication (Dellow and Lund, 1971; Sumi 1971). Also influencing mastication will be the higher centres of the brain such as the cerebral cortex so that the basic jaw-open, jaw-close pattern has added dimensions to integrate oral and dental sensory stimuli (Lund and Lamarre, 1973; Luschei and Goodwin, 1975; Sessle and Greenwood 1976). The coordination of the central and peripheral neural stimuli brings about the complex mandibular movements in mastication with respect to the

diet and food consistency in the various species. (Kay, 1978; Hiimae, 1978).

4.107 Kay and Hiimae (1974) noted that the manner in which the food is treated is a direct function of consistency of the food. The different animals they worked with, irrespective of their characteristic dental morphology, treated the same food substances in nearly an identical manner. Kay and Hiimae (1974), also imply that a given food consistency will require a particular approach to the opposition of teeth to cut up the food in the most efficient manner. Seligsohn and Szalay (1978) agree with that and suggest that an "innate-learned" recognition of the physical properties of food through mechanoreceptors in the teeth and mouth is probably a basic mechanism in mammals. Given that each species has a typically mammalian chewing cycle as well as genetically stringently controlled dental occlusion and a behavioural propensity for a particular diet, selection would rapidly maximise a tooth design most suited for the optimum division of a diet of a given consistency. With these phylogenetic possibilities, natural selection will favour those mechanical functions of the teeth which most efficiently divide preferred diets.

4.108 The dynamics of mastication in old world monkeys is similar to that observed in many other primates (Hiimae and Kay, 1973). A three stroke masticatory cycle

is present consisting of a preparatory stroke, which aligns the teeth **buccally** on the active side, a power stroke which titurates the food, and a recovery stroke, which returns the mandible to the beginning position (Kay 1978). During this activity, the pendulum undergoes tooth-food-tooth contact (puncture-crushing) as well as tooth-tooth interdigitation (chewing). Mills (1955) reported that Old World monkeys masticate differently than other primates in two ways. Firstly, during the buccal phase of occlusion there is an increased importance on the lingual cusps which are higher than the buccal ones, affording continual contact throughout the buccal phase and secondly during the lingual phase of occlusion, the mandibular buccal cusps pass between the upper lingual ones resulting in two facets on each cusp. The efficiency of this scissor-like mechanism is thought to be an adaptation to leaf and grass eating (Kay 1978).

4.109 One of the functions of normal attrition found in all mammals including primates is to maintain cutting edges of the teeth and probably occurs as the result of reflex grinding of the teeth in absence of food in the mouth (Miles, 1976). Functional attrition of teeth also depends on the texture of food, being more severe in diets with tough and abrasive food (Dubner et al., 1978). Attrition of teeth in malocclusion is more severe than normal (Miles, 1976).

4.110 It is understood that the peripheral sensory feedback from the teeth, muscles and temporomandibular joint has an important function in the modulation of jaw movements during the normal function of mastication (Sherrington, 1917; Thilander, 1973; Mercurio, 1981; Luschei and Goldberg, 1982). It is also apparent that normal functional movements of mastication are learned and an innate pattern established following eruption and occlusion of teeth (Thilander, 1973; Mohl, 1978; Dubner et al., 1978).

4.111 When considering the clinical aspects of the role of teeth in mastication, three main aspects need to be taken into account. Firstly, the loss of sensory feedback from the periodontal ligament and pulp due to loss of some or all the teeth and the loss of pulp in endodontically treated teeth. Secondly the changes in the occlusal relationships of opposing teeth due to restorative work such as fillings, crown and bridge and partial dentures. Thirdly, the condition of bruxism where there is involuntary unconscious grinding or clenching of teeth attributed to sensory feedback from non-harmonious tooth contacts (Kawamura 1974, Lundeen and Gibbs, 1982).

4.112 The degree of fine control exercised by the neuromuscular system in bringing the jaw into close proximity of occlusal contact (muscle control) versus

tooth control is an interesting but difficult phenomenon to explain. Hildebrand (1931) and Ai and Ishwara (1968) suggested that wear facets guide the chewing movements. Contradictory evidence has been obtained on the effect of occlusal feedback on mastication (Moller, 1966; Pameijer et al., 1968; Kavanagh and Zander, 1965). Unfavourable occlusal contact relations between the teeth of the opposing jaws have been thought to lead to avoidance responses serving to protect the teeth as well as the temporomandibular joint and musculature from trauma (Clayton, 1971; Hannam et al., 1977). However, complete anaesthesia of the dentition has been found not to change the mastication pattern (Schaerer et al., 1966). Experimental occlusal interference has also not changed the masticatory patterns recorded by EMG (DeBoever 1969). But alternatively, it has been found by Scharer et al. (1967), that occlusal interferences elicit reflex inhibition of mandibular movements. Shaerer et al. (1967) suggested that the mandible is guided by light irregular touching contacts into intercuspal position, moving within the boundaries of intercuspatation without sliding on it.

4.113 There is evidence that the neuromuscular system does appear to have capability for fine control when there is malocclusion (Clayton 1971; Dubner et al., 1978). The feedback from tooth contacts and the periodontal mechanoreceptors in normal mastication cycle

depends on a number of factors which will determine when a reflex response is obtained. Force and direction of stimuli in tooth contact is important. Alteration in sensitivity of the receptor threshold in conditions such as periodontitis or pulpitis may result in avoidance pattern (Dubner et al., 1978).

4.114 The question then arises as to what happens when some or all the teeth are lost, with small changes in the occlusion due to fillings, crown and bridge and partial dentures. In the adult, alterations in both position, loss of teeth, high fillings and other influence produce derangements of the memory system and thereby evoke learning of new masticatory patterns (Kolprogge and van Griethuysen, 1976). With time, however, these new jaw movements may contribute to dysfunctional states in components of the masticatory system which cannot adapt or compensate for the poor relationship of teeth (Lee, 1982). The condition of bruxism which involves involuntary grinding movements of the jaw are also thought to be due to poor occlusal relationship of teeth and nervous tension (Arnold, 1981; Mercurio, 1981; Lee, 1982). The other aspect is the control of occlusal relationship in individuals with malocclusion and those who have undergone orthodontic tooth movement. The neurophysiological control of mandibular movement through peripheral feedback appears insufficient in explaining as to why some people with occlusal problems experience

pain and discomfort while others have no symptoms. However, it is apparent that peripheral sensory feedback from teeth has an important role to play in normal functional mandibular movements and in any subsequent changes in occlusal relationship due to a filling and partial denture (Funakoshi et al., 1976; Lundeen and Gibbs, 1982).

4.115 The foregoing clearly bears directly on the importance of occlusal rehabilitation, which involves both, conscious and subconscious learning. This occurs through proprioceptive inputs from the position and morphology of the teeth as they relate to each other and to the mucosa of the lips, cheeks and tongue. (Kawamura, 1974; Kolprogge 1975). Thus, in occlusal rehabilitation of patients with discomfort in the muscles and temporomandibular joint or in the condition of bruxism, the occlusal relationship of anterior and posterior teeth must be maintained so as to provide harmonious muscle activity (Kahn, 1977, Lee, 1982).

4.116 It follows therefore that when a filling or any restoration is made in the teeth, or there is insertion of a crown and bridge or the partial denture, the aim is to maintain or restore the initial occlusal relationship. If the filling is high or the denture is ill-fitting, the sensitivity of the receptors of the periodontal ligament would detect any change in the occlusal relationship of



the teeth and perhaps lead to "avoidance" movements of the mandible. This in turn would cause discomfort to the patient (Kolprogge and van Guethuysen, 1976).

4.117 When some teeth are lost, the peripheral sensory feedback of the periodontal ligaments of the remaining teeth has been shown to be quite important in maintaining the efficiency of the muscular activity. The muscle activity in patients wearing over-dentures, where the periodontal ligament in two or three supportive teeth is intact, has been found to be more efficient than in patients with full dentures (Defranco, 1971, Ngasawa et al., 1979). The role of periodontium of remaining teeth in over-dentures has been considered to be important (Kay and Abes, 1976; Thayer, 1980).

4.118 In the edentulous state, when all teeth are lost, the sensory feedback from the periodontal ligament receptors is lost. As a result, the tactile sensibility, directional sensitivity, oral perception ability, and capacity to detect size of particles placed between the occlusal surface is greatly affected (Manly et al., 1952; Siirila and Laine, 1972; Christensen and Morimoto, 1977, Mohl and Drinnan, 1977).

4.119 There is a significant change in the mandibular posture following tooth loss and before insertion of the dentures. The mandibular position moves upwards and forwards (Richardson, 1980). The masticatory movements

in the edentulous patient are not harmonious, although the rhythmic movement is present. (Mohl and Drinnan, 1977). The receptors of the temporomandibular joint the muscles and mucous membrane play an important role in modulating the mandibular movements and perception of mandibular position as well as the discrimination of objects in the mouth in edentulous patients (Klineberg, 1971; Crum and Loiselle, 1972; Thilander, 1973; Kawamura, 1974; Mohl and Drinnan, 1977). The mechanoreceptors in the mucosa may be involved in the perception of mandibular position providing precise information as to the distribution of bite pressure (Atkinson and Ralph, 1973; Munakata, 1986). The mandibular movements in absence of some teeth or all teeth showed that the masticatory cycle was normal in cases where there were good dentures. This is because correct vertical occlusal relationship in the dentures produces normal mandibular movements (Atkinson and Shepherd 1973).

4.120 It has <sup>been</sup> debated whether teeth that have been endodontically treated may lose some sensory perception due to loss of pulp and whether or not these teeth may be used as abutment teeth in fixed bridges (Dubner et al., 1978). The treatment of anterior as well as posterior teeth is a recommended practice in order to preserve the integrity of the dental arch and retain the periodontal sensory feedback which has been shown to be significant in modulation of jaw movement. Apicectomies, the

practice to treat the root apex and leave the rest of the root intact following endodontic treatment is carried out in cases where a routine root canal treatment is not successful. This would retain the sensory function of the periodontal ligament.

4.121 The role of oral perception and proprioception and its significance to prosthodontics has been considered extensively by Crum and Loiselle (1972). The masticatory system must be considered as an integrated system made up of various components and the importance of preserving teeth is apparent for maintaining efficient masticatory function.

4.122 The present study in the primates has shown that incisors, canines and molars have proprioceptive connections to the mesencephalic nucleus while incisors and canines are well represented and molars sparsely represented in the trigeminal ganglion. It is suggested that anterior teeth ensure good occlusal relationship and anterior guidance during jaw movement and impart greater sensory perception. The molars, on the other hand may provide occlusal stops, maintaining vertical relationship and prevention of large occlusal forces due to inhibitory reflexes, at the same time protecting the joint. In addition loss of anterior teeth may result in derangement of mandibular movement and posterior teeth may result in undue stresses in the temporomandibular joint and muscles during occlusal phase of chewing.

#### E. Conclusions and Suggestions for Further Studies

4.123 The present study in the vervet monkey and olive baboon using HRP retrograde tracing method has shown that the periodontal ligament and gingivae of maxillary and mandibular incisors, canines and molars have ipsilateral afferent neural connections mainly with the mesencephalic neurons located in the caudal part of the mesencephalic nucleus of trigeminal nerve. The incisor teeth have a significantly higher number of mesencephalic neuron connections than either the canine or molar teeth. The dental pulp has no mesencephalic afferent neural connections.

4.124 The periodontal ligament and gingivae of the maxillary and mandibular incisors and canines have a large and preponderantly ipsilateral afferent neuron representation in the trigeminal ganglion compared to the molars which have a sparse ipsilateral representation in the trigeminal ganglion. There are significantly more periodontal trigeminal ganglion neurons innervating the anterior teeth than the molar teeth. The afferent neurons of the dental pulps of the various teeth types are also located in the trigeminal ganglion, the incisor tooth pulps with larger number of neuron connections than the molars.

4.125 The present study has shown that the periodontal ligament has dual afferent connections. Firstly, are the afferents of the mesencephalic neurons, with mechanoreceptors which are thought to be mainly rapidly adapting high threshold receptors subserving proprioceptive modality. Secondly are the afferents of the trigeminal ganglion neurons with mechanoreceptors which are thought to be mainly slowly adapting, low threshold receptors. However, both types of neurons, with receptors which are slowly and rapidly adapting are found in the mesencephalic nucleus and the trigeminal ganglion (Ness, 1954; Linden, 1978; Appenteng et al; 1982). The trigeminal ganglion neurons also have free nerve endings which together with the mechanoreceptors, subserve the modalities of general sensations such as pain, pressure, touch and temperature.

4.126 The proprioceptive connections of the periodontal ligaments of the incisors, canines and molars to the mesencephalic nucleus suggests that the anterior teeth as well as posterior teeth are important in mediation of masticatory functions. Since the incisors are seen to have more neural connections to the mesencephalic nucleus and the trigeminal ganglion, it is plausible that they may be functionally more significant and be involved, particularly in the anterior guidance of the jaw during the early closing movements of the jaw. The incisors may also be involved in perceiving the sensory forces on

teeth, discrimination of size and consistency of food and reflex positioning the food bolus in the mouth. The molar teeth, with more definite neural connections to the mesencephalic nucleus than the trigeminal ganglion, presumably have more high threshold rapidly adapting receptors which may be activated in the later stages of the occlusal phase. The tooth-food and tooth-food-tooth contact may stimulate the periodontal receptors and initiate the reflex guiding movements of the jaw to achieve grinding and chewing of the food and possibly to provide information on the magnitude of forces.

4.127 In the vervet monkey and olive baboon, although the canine is a large and prominent tooth, it has been found in the present study not to have more neural connections to the mesencephalic nucleus and trigeminal ganglion than the smaller incisor teeth. The fairly large neural connections of the canine to the trigeminal ganglion may be involved in perceiving sensory forces on the teeth, especially during lateral jaw movements. The moderate mesencephalic neural connections of the canine in these Old World monkeys suggest that the prominent canine does not have as essential a role in mastication of mainly leaf, grass, fruit and nuts. The large canine in the monkey and baboon may be an organ for offence and defence. On the other hand, in the carnivorous cat and dog, the canine has been shown to be highly sensitive and well represented in both the trigeminal ganglion and

mesencephalic nucleus, (Hannam, 1969 a,b; Linden, 1978); and appears to have an essential role in the mastication of meat.

4.128 The caudal location of the periodontal mesencephalic afferents in the vervet monkey and olive baboon found in this study agrees with similar findings in the cat, rat and rabbit (Cody et al., 1974; Linden, 1978; Jacquin et al., 1983 b ; Passatore et al., 1983; Capra et al., 1984, and Gottlieb et al., 1984). It is of interest to note that the periodontal mesencephalic afferent neurons are segregated caudally, while the muscle spindle afferent neurons of jaw elevator muscles are distributed along the rostro-caudal extent of the nucleus in the cat, rat, rabbit and monkey (Ibrahim and Leong, 1979; Passatore et al., 1983; Capra et al., 1985). The caudal location of the periodontal afferents could be to allow closer proximity to the target organs, the teeth, to the supratrigeminal nucleus and motor nucleus of trigeminal nerve for reflex connections, to the cerebellum and to other sensory and reticular neurons Corbin and Harrison, 1940; Elias and Taylor, 1984; Elias et al., 1985; Capra et al., 1985 and Rokx et al., 1986 a,b)

4.129 The clustering of some 2 to 9 mesencephalic neurons, with some of the neurons within the clusters in soma-soma contact has been observed in the present study



in the monkey and baboon. Similar observations have been made in the rat and cat (Hinrichsen and Larramendi, 1970; Nomura et al., 1985). The clustering of cells with soma-soma contact is of possible functional significance in that the afferent input from one tooth may be electrotonically coupled to other periodontal neurons and also to muscle spindle neurons so as to synchronize the activity of the motor neurons. (Passatore et al., 1983, Nomura et al., 1985). One or two HRP labelled periodontal mesencephalic neurons adjacent to unlabelled neuron(s) in a cluster were observed in the present study and by Gottlieb et al. (1984). One or more mesencephalic muscle spindle HRP labelled neurons close to unlabelled cells in cluster were also observed by Gottlieb et al. (1984), and Capra et al. (1985). This evidence suggests that a cluster may have the periodontal and muscle spindle afferent neurons, afferent neurons of various teeth, or those of various muscles. Thus, a cluster of mesencephalic neurons may act as a functional unit so that activity occurring in a single cell of the unit may modulate the activity of the entire unit.

4.130 It has been observed in this study that there was faint bilateral labelling of neurons in the most caudal region of the mesencephalic nucleus near the motor nucleus, presumably in the region of the supratrigeminal nucleus. It is suggested from these observations that the ipsilateral mesencephalic neurons may project to the

interneurons of the supratrigeminal nucleus which may modulate the reflex connections to the jaw-opening and jaw-closing motor neurons as well as co-ordinate bilateral oral behaviour during mastication. The preponderantly ipsilateral trigeminal ganglion connections of the teeth to the motor neurons may be modulated through the interneurons of the sensory nuclei or possibly the supratrigeminal nuclei for the co-ordination of bilateral movements of the jaw.

4.131 It has been shown in the present study that the discrete periodontal, gingival and pulpal afferent neurons from various maxillary and mandibular teeth have a somatotopic distribution within the trigeminal ganglion with respect to the maxillary and mandibular compartments. The HRP labelled afferent neurons innervating mandibular teeth are found in the postero-lateral aspect of the ganglion and those of the maxillary teeth are found in the middle of the ganglion, both along the dorso-ventral extent. There was no localization of the afferent neurons of the individual teeth types within the respective maxillary and mandibular compartments of the ganglion. These findings in the monkey and baboon agree with those of others in the cat, rat and monkey (Kerr and Lysak, 1964; Beaudreau and Jerge, 1968; Lende and Poulous, 1970; Marfurt 1981 a., Jacquin et al., 1983 a)

4.132 The quantification of the mesencephalic neurons and the trigeminal ganglion neurons using the methods of cell counts and stereology has shown that in the mesencephalic nucleus of the monkey there are  $1379 \pm 362$  to 2674 cells and the baboon there are  $1620 \pm 366$  to 2816 cells (bilaterally). In the trigeminal ganglion of the monkey there are about 98073 to 101178 cells and in that of the baboon there are 137250 to 153555 cells. In the present study variations were observed in the cell counts between the mesencephalic nuclei and between the ganglia of the various monkeys and baboons respectively. It appears from the analysis, that about 10%-15% of the mesencephalic neurons (unilaterally) and 0.32% to 0.58% of trigeminal ganglion neurons in the baboon and monkey have afferent connections with the incisors, canines and molar periodontal ligament and gingival receptors.

4.133 To gain a better understanding of the sensory connections and morphofunctional aspects of the teeth, some further studies need to be conducted. Studies on the afferent connections of the periodontal ligament and pulp of premolar teeth in the monkey and baboon would be of interest to determine the quantitative neuron representation with respect to that of anterior teeth and molar teeth. Studies on the quantitative analysis of the nerve fibres in the periodontal ligaments of the various teeth types would also show whether there are any

differences in the density of innervation of the various teeth types.

4.134 More studies of pulpal connections to the mesencephalic nucleus and trigeminal ganglion with a larger sample of selected teeth types could be carried out. The cavity depth, the volume of the HRP solution used and non-leakage of the tracer to the periodontal ligament through the lateral canals and periapically should be controlled and ascertained in these studies.

4.135 The functional significance of the clusters of mesencephalic neurons may be elucidated by using two retrograde tracers such as HRP injected into the muscles of mastication and another tracer such as DAPI into the periodontal ligament. The study may show the relationship of the labelled muscle spindle and periodontal afferent neurons, especially in the clusters of neurons. Furthermore, electron microscopic studies of mesencephalic neurons and the clusters, using radioactive and other labelled tracers may also provide a clearer understanding of the synapses, soma-soma and axo-somatic contacts of these neurons.

4.136 The possible bilateral connections of the dental afferents to the supratrigeminal nuclei, presumably along the collaterals of the mesencephalic neurons may be confirmed by using HRP-WGA (HRP-wheat germ agglutinin

complex) which is a better tracer for anterograde central connections of neurons (Mesulam, 1982)

4.137 The quantification of total number of mesencephalic nucleus and trigeminal ganglion neurons in the monkey and baboon using a more recent method of dissector (selector) for estimation of particle numbers in structures irrespective of the section thickness, particle size, shape and orientation (Cruz-Orive 1985, Gundersen 1986), would be of interest to see how the new data compares with that of the present study. The quantification of the neurons of the mesencephalic nuclei and the trigeminal ganglia of other animals such as rabbit, rat, cat, frog, goat, crocodile and man, using stereological methods would show how the new data compares with known data obtained using other methods. The data could also be used for comparing the total number of neurons in the mesencephalic nuclei and trigeminal ganglia of animals with different body size, masticatory behaviour and diet (Weinberg, 1928; Du Brul, 1960).

4.138 Further studies on the quantification and somatotopic mapping of the dental afferents of the various teeth types in the mesencephalic nuclei and trigeminal ganglia in some animals such as the cat, rat, goat and other non-human primates using HRP or other retrograde tracing methods might show the functional

importance of the various teeth types in animals with different dentition, diet and masticatory jaw movements. Moreover, these studies might well enhance the understanding of the role of peripheral feedback from the various teeth types in the regulation of the complex masticatory behaviour in man.

4.139 In conclusion, the present study in the monkey and baboon has shown that the afferent connections of the various teeth types have a differential quantitative representation in the mesencephalic nucleus and the trigeminal ganglion. The proprioceptive and sensory connections of teeth suggests that the peripheral feedback from the teeth has an important role in the function of mastication and sensory perception of food and tactile forces on the teeth.

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